# JOURNAL OF DAIRY SCIENCE

## Contents


*An Activity Test for Cheddar and Cottage Cheese Starters.* B. E. HORRALL AND P. R. ELLIKER ..... 245

*The Use of Reconstituted Non-fat Dry Milk Solids for Propagating Mother and Batch Starter Cultures.* B. E. HORRALL AND P. R. ELLIKER ..... 250

*The Relationships among Cracked Soybeans Fed, Barn Temperature and the Degree of Unsaturation of Milk Fat.* J. B. FRYE, JR., C. Y. CANNON AND E. W. BIRD ..... 257

*The Relation between the Degree of Solidification of Fat in Cream and its Churning Time. II. The Physical Distribution of the Liquid-solid Phases within the Globule.* J. ROBERT BRUNNER AND E. L. JACK ..... 267

*Electrophoresis of Milk Proteins. I. Some Comparisons of Salt-acid and Salt-lyophilized Whey Fractions.* WILLIAM G. STANLEY, A. C. ANDREWS AND C. H. WHITNAH ..... 275

*Stale-flavor Components in Dried Whole Milk. III. The Steam Distillation of Stale-flavor Components from Stale Butter Oil.* R. MCL. WHITNEY, KATHERINE PAULSON AND P. H. TRACY ..... 281

*Importance of Hay Quality as Indicated by Feeding Trials with Identical Twin Dairy Heifers.* CHANDRAKANT N. KELKAR AND T. W. GULLICKSON 288

*The Number of Proved Sons Necessary to Evaluate the Transmitting Ability of a Dairy Sire.* W. E. WASHBON AND W. J. TYLER ..... 293

*Flavor Deterioration Associated with the Lipid Phase of Whole Milk Powder.* ARTHUR T. MUSSET, STUART PATTON AND CHESTER D. DAHLE ..... 299

*The Use of Dehydrated Forages in Dairy Cattle Rations. I. Grain Substitution with Finely Ground Material.* E. B. HOPE, R. E. ERB, T. H. BLOSSER, U. S. ASHWORTH AND A. O. SHAW ..... 306

*The Effect of Vitamin A from Prenatal Storage and from Ingestion of Colostrum on the Neonatal Calf.* L. NEZVESKY, H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, C. I. BLISS AND A. A. SPIELMAN ..... 315

*Studies of Heated Milk. I. Formation of 5-Hydroxymethyl-2-Furfural.* STUART PATTON ..... 324

*Parturient Paresis. VI. Some Changes in the Urinary Excretion of Certain Constituents at Parturition and their Possible Association with Changes in the Blood Picture.* T. H. BLOSSER AND VEARL R. SMITH ..... 329

*Changes in Milk Production with Age and Milking Frequency.* JAY L. LUSH AND ROBERT R. SHRODE ..... 338

*Program, Forty-fifth Annual Meeting* ..... 358

*Abstracts of Literature* ..... A67



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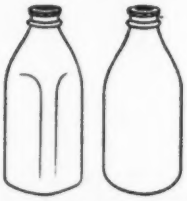

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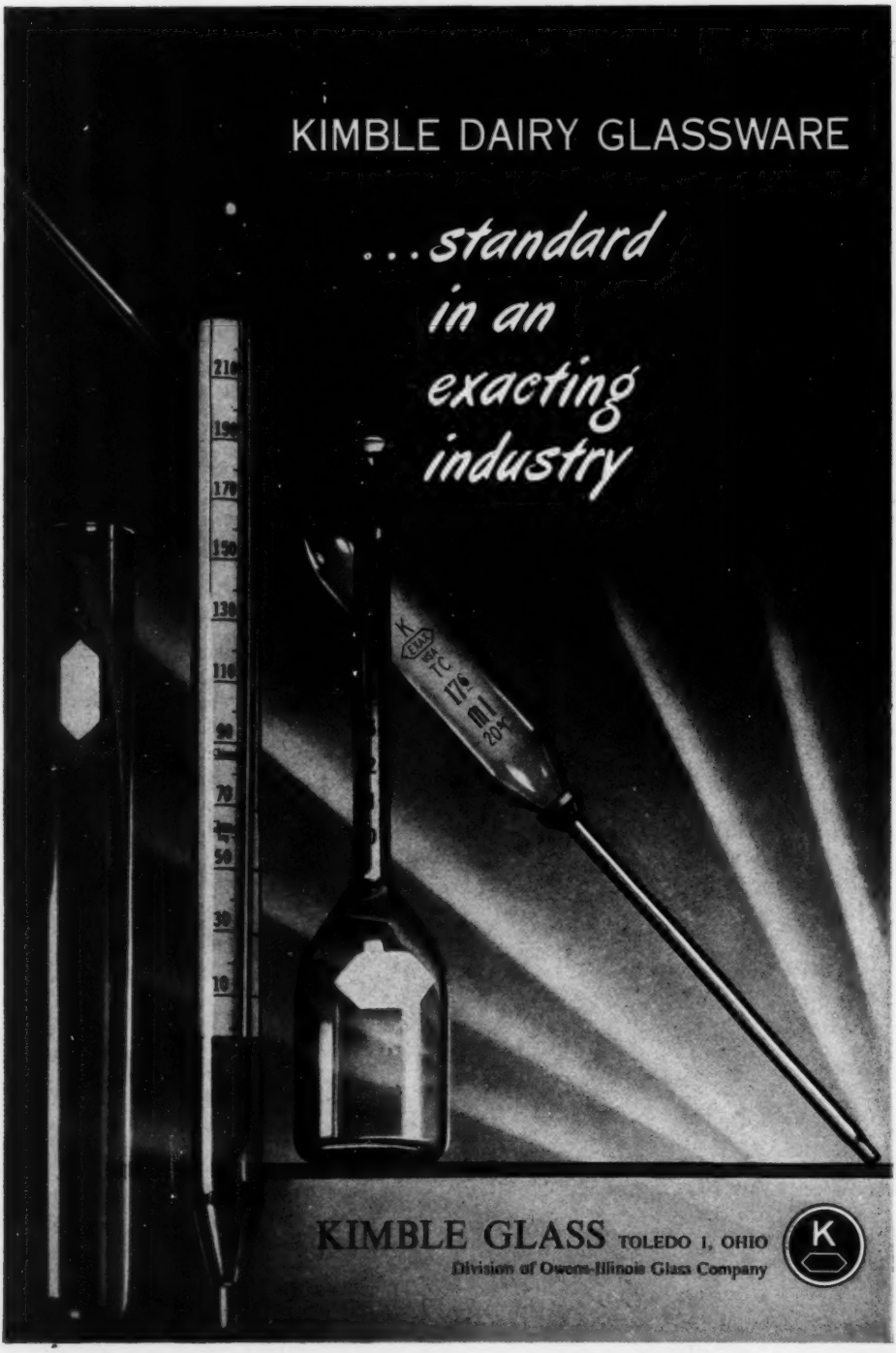


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JOURNAL OF DAIRY SCIENCE

VOLUME XXXIII MAY, 1950 NUMBER 5

# AN ACTIVITY TEST FOR CHEDDAR AND COTTAGE CHEESE STARTERS[1]

B. E. HORRALL[2] AND P. R. ELLIKER[3]

*Purdue Agricultural Experiment Station, West Lafayette, Indiana*

Preliminary studies on slow acid development in the manufacture of cheddar cheese in Indiana demonstrated the necessity, in a plant starter program, for a simple, accurate test to determine the activity of starter cultures under conditions simulating those in the cheese vat. This report deals with the development of a plant test to determine activity of cheddar and cottage cheese starter cultures.

High activity in starter cultures appears to be of more importance today than in the past because of the general trend toward pasteurization of milk for cheddar cheese manufacture and consequent destruction of acid-producing organisms normally present in the raw milk.

## REVIEW OF LITERATURE

Whitehead and Cox (4) devised a method of testing the vitality of starters by simulating the cheese-making process in pint jars and testing the acidity of the whey at various intervals. This method or modifications of it have been used by a number of laboratories in this country.

Elliker and Frazier (2) determined the activity of Swiss cheese starter cultures by growing them at temperatures near their maximum in sterile reconstituted skim milk prepared from the same lot of powder. The rate of growth was determined by direct microscopic counts of living cells and also by determinations of pH and titratable acidity at hourly intervals over an 8 hr. period.

Anderson and Meanwell (1) measured the activity of cheddar cheese starters by adding a 1 per cent inoculum of starter to sterilized milk and incubating duplicate tubes for 6 hr. at 30 and/or 37° C. The acidity then was determined with 0.11 *N* NaOH using 1 ml. of 0.5 per cent phenolphthalein indicator per 10 ml. of the incubated milk.

Johns and Berard (3) in studying relationship between overripening and starter activity, modified the Whitehead and Cox vitality test. Fresh sterile skim milk was inoculated with 1 per cent of the culture. The samples were incubated in a water bath at 86° F. for 2 hr. and then transferred to an incubator

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[1] This study was made possible by a special project grant from Kraft Foods Co., Chicago, Illinois.

[2] Now with Kraft Foods Co., Chicago, Illinois.

[3] Present address: Department of Bacteriology, Oregon State College, Corvallis.



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at 102° F. where they were kept for the next 4 hr. The samples were mixed carefully before sampling and were titrated for acidity every hour.

## EXPERIMENTAL

A survey was made of activity tests for cheddar cheese starter cultures. Certain of the methods were rather time consuming and difficulty was experienced in obtaining uniform results by methods employed in the average cheese plant. Consequently an attempt was made to develop a method of determining activity of cheese starter cultures that would be simple, rapid and yet consistent. It also was believed that the method should involve a test of a culture's ability to develop at or near the cooking temperature employed in the manufacture of cheddar cheese.

*Procedure for activity test developed.* The test finally developed for activity of mother cultures and starters employs a reconstituted high grade, spray, non-fat dry milk solids for the culture medium. The use of whole milk from a selected herd as a medium for the activity test was attempted, but the results were less consistent than with the reconstituted milk. It was believed that if the same lot of milk powder should be used over a long period of time, one variable factor would be eliminated. The reconstituted milk was prepared at the rate of 10 per cent of the non-fat dry milk solids in distilled water and was heated in flasks at 15 lb. pressure for 10 min. Respective 10-ml. quantities of sterile milk were pipetted aseptically into sterile screw-top test tubes and adjusted to 37.8° C. in a water bath. Each tube was inoculated with 0.3 ml. of the starter culture to be tested and incubated at 37.8° C. for 3.5 hr. Then the entire contents of the tube, together with 5 ml. of distilled water used to rinse the tube, were titrated with 0.1 *N* NaOH to a faint pink color using phenolphthalein as an indicator. The results were recorded as per cent lactic acid. This figure was termed the activity factor of the culture.

The test outlined above, or an earlier modification which employed an incubation time of 6 hr. and a 1 per cent inoculation, has been used daily in 20 plants for a period of more than 4 yr. It has aided materially in selecting starters and cultures to be used for cheese manufacture.

*Comparative results with proposed activity test.* Figure 1 represents a comparison of the proposed test for culture activity and the Whitehead and Cox test since the latter method has been most commonly used in the past to determine activity of cheddar cheese cultures.

Many comparisons using these methods were made on mother and batch starters. One representative comparison on the mother culture used to inoculate the batch culture subsequently employed in the cheese vat is shown in figure 1. The milling acidity shown represents the rate of acid production in the cheese by the batch starter organisms inoculated with the respective mother culture. The other two comparisons shown in figure 1 represented activity tests on batch cultures used to inoculate cheese milk.

The points plotted for the Whitehead and Cox test were the acidity of the whey from the third or last draining which was taken at the end of 6.5 hr.

These points represented the average of duplicate tests. The original method of Whitehead and Cox involves an estimation of the activity based on the difference between the last two titrations. The original method was followed, but for purpose of convenience only the last titration is included here. The milk used for the Whitehead and Cox test was whole milk from a selected herd. This test milk was pasteurized at 65.6° C. for 5 min.

The new activity test was made in duplicate and the points plotted repre-

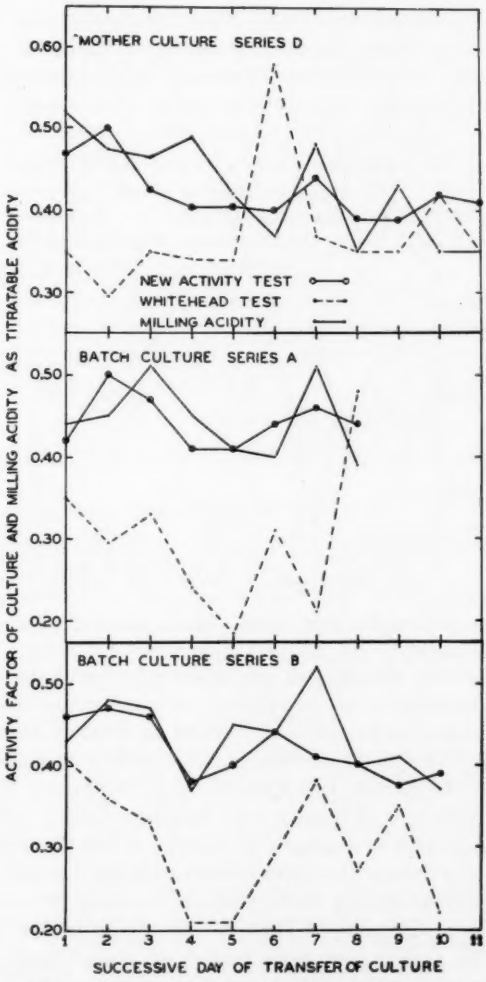
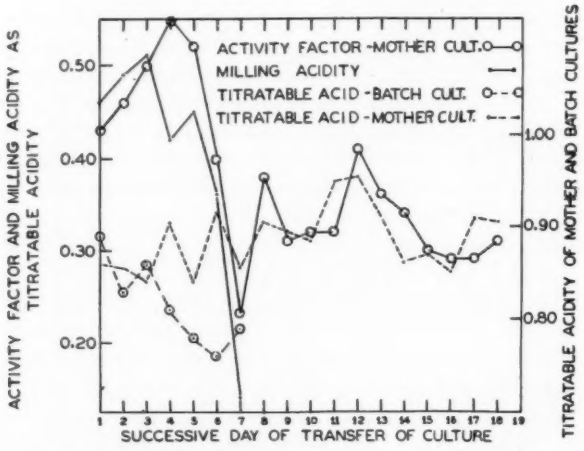



FIG. 1. Comparison of proposed activity test, Whitehead and Cox test and milling acidity of the cheese. (Milling acidity for series D necessarily represents one transfer removed from mother culture tested.)

sented an average of the results. It was found that duplicate and triplicate titrations with the Whitehead and Cox test varied considerably. One duplicate test varied 0.3 per cent and others varied as much as 0.1 per cent at the third drain, while duplicate tests with the new method varied only 0.04 per cent as a maximum. Most of the tests showed less than 0.02 per cent variation.

The batch starter (series A and B) was made with whole milk in 10-gal. cans, heated to 87.8° C. and held for 1 hr. The amount of starter used in the cheese vat was 0.7 per cent.

The curves on batch cultures series A and B suggest that the new activity test predicted more accurately the milling acidity of the cheese which is an indication of the activity of the starter. However, it is realized that the proposed test will not always predict the milling acidity of the cheese because of the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TITRATABLE ACIDITY OF MOTHER AND BATCH CULTURES
ACTIVITY FACTOR -MOTHER CULT.
MILLING ACIDITY
TITRATABLE ACID -BATCH CULT.
TITRATABLE ACID -MOTHER CULT.
0.50
0.40
0.30
0.20
1.00
0.90
0.80
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SUCCESSIVE DAY OF TRANSFER OF CULTURE

FIG. 2. Activity of one culture over a period of 18 days.

effect of such factors as variation in rate of inoculation of the vat milk, quality of the milk used, presence of bacteriophage and overheating in the vat. Starter cultures that developed sufficient acid to yield an activity factor of more than 0.35 in the activity test usually resulted in cultures that were active in the cheese vat, providing all the factors listed above were eliminated. Starters with an activity factor of 0.30 to 0.35 usually were found to develop acid more slowly in the cheese vat. A starter with an activity factor of less than 0.30 usually developed little or no acid during the cheese-manufacturing process.

*Correlation between activity factor and daily activity of successive transfers of a mother culture.* Records of activity tests made in two cheese plants on mother cultures for a period of more than 4 yr. reveal that the new test frequently indicates those cultures that are deteriorating. The results with one culture are shown in figure 2, which gives the activity factor of the mother culture, the acidity of the mother culture at the time it was transferred, the acidity

of the subsequent batch culture and milling acidity when used for cheese manufacture.

This culture at the beginning of the trial was high in activity and produced the desirable milling acid with 0.6 to 0.7 per cent inoculation in the cheese vat. It had been used in the plant about 6 wk. before any change was detected. It will be noted in figure 2 that the activity of the culture was high (0.56); then it rapidly decreased and in three transfers had an activity factor of 0.23, which is considered an inactive culture. The milling acidity of the cheese also decreased each day after the culture started to lose activity. On the last day that the culture was used in the cheese vat, the mother culture had an activity factor of 0.23 and the vat milled at 0.14 per cent acid. The propagation of the culture was continued for 11 days to revive it. On the seventh and eleventh days (as shown in figure 2), the inoculation was increased. In both instances the activity increased the following day but dropped again the next day. As shown by the activity test, the culture did not regain its earlier high activity during the 18-day period. The results also indicate that the new activity test is more sensitive in predicting inactivity than daily determination of titratable acidity of mother and batch cultures.

The new test has been used to determine the activity of multiple as well as single-strain cultures employed in cheddar and cottage cheese. It has proved a valuable aid in reducing incidence of slow acid production in the cheddar as well as cottage cheese making process.

## SUMMARY

1. A simple, rapid test for activity of cheddar and cottage cheese cultures is described.

2. The method involves inoculation of 3 per cent of the starter culture to an autoclaved reconstituted non-fat dry milk solids medium and titration of acidity after 3.5 hr. incubation at 37.8° C.

3. The results indicate that the new activity test is reasonably accurate and consistent and is sufficiently simple to be applied readily to plant conditions. It has been employed in a number of cheddar cheese plants for more than 4 yr. and has been a valuable aid in reducing incidence of slow acid production.

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# THE USE OF RECONSTITUTED NON-FAT DRY MILK SOLIDS FOR PROPAGATING MOTHER AND BATCH STARTER CULTURES[1]

B. E. HORRALL[2] AND P. R. ELLIKER[3]
*Purdue Agricultural Experiment Station, West Fafayette, Indiana*

AND

GEORGE KENSLER
*Kraft Foods Co., Chicago, Illinois*

The importance of high activity in lactic starters since the introduction of pasteurization of milk for cheddar cheese generally is acknowledged. It also is known that the milk used to grow the mother and batch starters must be of the highest quality for consistently high starter activity. Whole milk usually has been used most frequently for propagating cheddar cheese starters. In some cases the mixed milk as it arrives at the plant has been used. Skim milk and whey have been employed less frequently.

Another recognized fact is that milk from individual herds varies from day to day in its ability to support growth of starter bacteria. Such variations affect the uniformity of starter activity. It was believed that if mother cultures and starters could be carried in milk of the same composition over long periods of time, more uniformity in their activity could be obtained. This report represents results of more than 4 yr. of study on the use of reconstituted spray dried, non-fat milk solids for preparation of mother cultures and starters in commercial cheese plants.

## EXPERIMENTAL

*Concentration of solids in reconstituted milk.* Preliminary studies showed that a 10 per cent concentration of spray, non-fat dry milk solids in distilled water yielded as satisfactory cultures as did 12, 15 and 18 per cent concentrations.

*Activity of mother cultures carried in reconstituted and selected herd milk.* A comparison was made of the daily activities of a mother culture carried in 10 per cent high grade, spray, non-fat dry milk solids in distilled water and in selected whole milk from an individual herd. Two hundred ml. of the reconstituted milk and whole milk were placed in respective 500-ml. erlenmeyer flasks, autoclaved at 15 lb. pressure for 10 min., cooled to 21.1° C. and inoculated with 0.7 per cent of an active culture. These milks were incubated at 21.1° C. for 15 hr. and then cooled in ice water. The activities of these cultures were tested by the method described in a preceding paper (1).

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[1] This study was made possible by a special project grant from Kraft Foods Co., Chicago, Ill.

[2] Now with Kraft Foods Co., Chicago, Ill.

[3] Present address: Department of Bacteriology, Oregon State College, Corvallis.

Results representative of numerous trials are shown in figures 1 and 2. The activity of the mother culture propagated in non-fat dry milk solids was relatively constant from day to day (the lowest activity factor was 0.40 and the highest, 0.48) while that of the whole milk varied from 0.34 to 0.51, with a slightly lower average activity factor than the reconstituted skim milk. Other trials conducted in the same manner but at different seasons also showed that

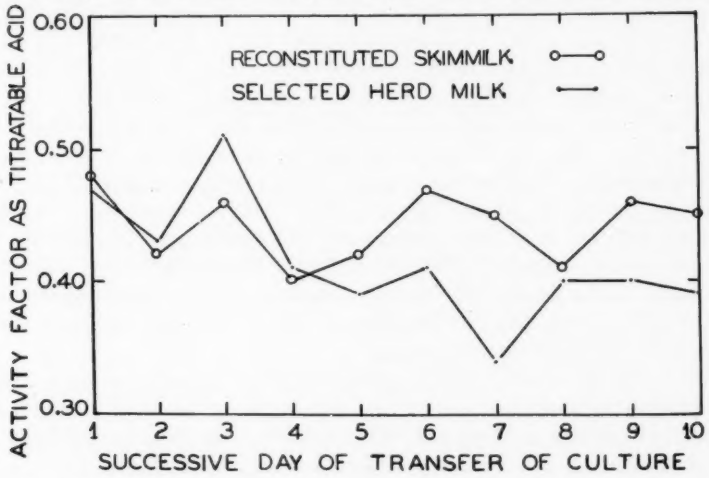


FIG. 1. Activity of mother cultures propagated in reconstituted 10 per cent spray, non-fat dry milk solids and in selected whole milk.

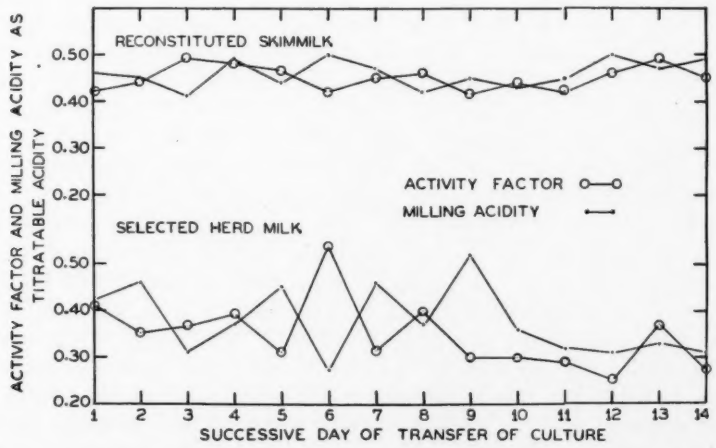


FIG. 2. Comparison of the activity of batch starters propagated in reconstituted skim milk and selected whole milk and the respective milling acidities of cheeses prepared from these starters.

the whole milk cultures usually had an average activity factor lower than that of the reconstituted, non-fat, dry milk solids cultures. In all of these trials the acidities of the whole milk cultures at the end of the daily incubation period of 21.1° C. averaged from 0.1 to 0.2 per cent lower than those of the cultures propagated in the reconstituted milk.

*Activity of batch starters prepared with reconstituted and selected whole milk.* A study next was made of activity of batch starters prepared in reconstituted, spray, non-fat dry milk and in selected whole milk. The whole milk was from the same herd as that used in the previous experiment on mother cultures. The milks were pasteurized at 87.8° C. for 1 hr. in cans, then cooled to 21.1° C., inoculated with 0.7 per cent of an active lactic culture and incubated at this temperature for 15 hr. The rate of inoculation of the vat milk was 70 lb. of starter to 10,000 lb. of milk. The cheese was made by the precision method (2).

Results in figure 2 indicate that the starters carried in the reconstituted milk were more constant in activity than those in the whole milk. The activity factors of the starters propagated in the reconstituted milk ranged from 0.415 to 0.49, while those of the whole milk varied from 0.25 to 0.54; the respective milling acidities varied from 0.41 to 0.50 per cent for the reconstituted milk and from 0.27 to 0.52 for the whole milk starter.

Mother cultures, both multiple and single strain, have been carried continuously in the laboratory in reconstituted non-fat dry milk solids for more than 4 yr. and both mother cultures and batch starters have been propagated by this method in 20 plants during this period with satisfactory results.

*Influence of type of water used in reconstituting milk for mother and batch starters.* The large quantities of distilled water that were required for preparing the batch starter presented a problem. An experience with one plant early in the studies emphasized the importance of the type of water used for reconstituting the milk solids for starter milk. Following a preliminary failure of cultures grown in milk reconstituted with this plant water, a study was carried out on the activity of cultures grown in milks reconstituted with the plant water and with distilled water. In the trial, milks were pasteurized in four 10-gal. cans, (two cans for each reconstituted milk) at 87.8° C. for 1 hr., cooled to 21.1° C. and inoculated with 0.75 per cent culture. The cultures in milk made with the plant water (which in this case was from the city supply), after 15 hr. incubation, had activity factors of 0.23 and 0.27. The controls grown for the same period in milk reconstituted with distilled water had activity factors of 0.45 and 0.48. Since the activity factor was low on the plant water starter, 105 lb. were added in 10,000 lb. of pasteurized vat milk, whereas, only 75 lb. of the distilled water starter were used for the same quantity of milk. The acidity of the wheys 4.5 hr. after setting the milk were 0.25 per cent for the cheese made with plant water culture and 0.49 for that made with the distilled water culture. The batch starter prepared with the plant water milk produced acid normally during its incubation period at 21.1° C. and except for the activity test would have been judged as active for cheese manufacture as that made with distilled water. Following this experiment, an attempt was made to determine why use of plant

water resulted in an inactive starter. The solids content of the water was found to be 0.19 per cent, which is considered quite high. No specific toxic factor was found but occasional subsequent use for mother cultures always resulted in an inactive culture.

Water from the plant well also was available in this plant and a culture activity comparison was made of whole milk and milks reconstituted with distilled water, the city water and with plant well water. The activity factor was determined by the activity test. Tubes of each milk were inoculated and carried with daily transfers in triplicate and the activity tests repeated on 6 successive days. The city and well waters were drawn each day to prepare the reconstituted milks. The whole milk came from the same patron throughout the trial.

TABLE 1

*Activity factors of starter cultures in selected whole milk and in milks reconstituted with city water and plant well water*

| Milk reconstituted w/city water | | Milk reconstituted w/plant well water | | Milk reconstituted w/distilled water | | Whole milk | |
|---|---|---|---|---|---|---|---|
| Control[a] | Inoculated | Control[a] | Inoculated | Control[a] | Inoculated | Control[a] | Inoculated |
| 0.19 | 0.22 | 0.18 | 0.47 | 0.18 | 0.46 | 0.20 | 0.43 |
| 0.19 | 0.21 | 0.18 | 0.44 | 0.18 | 0.45 | 0.21 | 0.44 |
| 0.19 | 0.22 | 0.19 | 0.48 | 0.18 | 0.47 | 0.20 | 0.40 |
| 0.19 | 0.24 | 0.18 | 0.48 | 0.185 | 0.49 | 0.22 | 0.41 |
| 0.18 | 0.21 | 0.18 | 0.46 | 0.18 | 0.47 | 0.21 | 0.39 |
| 0.18 | 0.21 | 0.18 | 0.47 | 0.18 | 0.47 | 0.20 | 0.35 |

[a] Control represents uninoculated, incubated milk.

The results shown in table 1 indicate that the plant well water was satisfactory for preparation of the reconstituted milk but the city water again resulted in a milk that failed to produce an active culture. The whole milk yielded active cultures for the most part, but the cultures were less uniform in activity in the whole milk than the milks reconstituted with distilled and plant well waters. The trial differentiated sharply between the milk prepared with city water and the other milks. It emphasized the need for preliminary trials in the form of culture activity tests on milks reconstituted from various waters before they can be employed for preparing reconstituted starter milks. In another plant both the city and plant well water failed to produce active cultures when used to reconstitute the milk powder. Waters from several farm wells in the same vicinity were tested and found to produce less active cultures than when distilled water was used for reconstituting the starter milks. The water from one farm well about 0.5 mi. from the plant provided an active starter culture.

*Method of testing suitability of milk powders for preparation of starter milks.* Experience with various lots of powder has emphasized the importance of high grade, non-fat dry milk solids in preparing the starter milk and has shown that each barrel of powder must be tested to insure that the powder will produce a culture with high activity. The following method has been devised for testing the suitability of non-fat, dry milk solids for this purpose: (a) Weigh 10-g. portions of the unknown powder to be tested and of a powder whose ability to

support growth is known. Place the portions of unknown and known powder in respective flasks. (b) Measure 90 ml. of distilled water and add to each powder. Mix until free from lumps. (c) Measure exactly 10-ml. portions of the above milks into screw-cap test tubes. Prepare five tubes of the unknown and five of the known reconstituted milks. (d) Sterilize at 15 lb. pressure for 10 min. (e) Cool to 37.8° C. (Tubes not to be used immediately should be refrigerated.). (f) Inoculate one tube each of the unknown and known reconstituted milks with 0.3 ml. of an active culture. (g) Incubate in water bath at 37.8° C. for 3.5 hr. together with an uninoculated control tube of each milk. (h) At the end of the incubation period cool and determine titratable acidity on the entire contents of the tube plus 5 ml. of distilled water used to rinse the tube. Record as activity factor. (i) On the same day, inoculate one tube of the unknown milk with 0.1 ml. of the same active culture for the first serial transfer and incubate at 21.1° C. for 15 hr. Repeat for 2 successive days (three serial transfers). (j) Make activity test on each transfer at the end of incubation period.

In table 2 it will be noted that barrels 2, 18 and 34 showed higher activity factors than the known powder and the activity of the cultures did not decrease when they were serially transferred for 3 successive days in milk prepared from those barrels. Barrels 42 and 43 showed lower activity factors than the known powder. The last two barrels listed were not used for preparation of starter milk in the plant because of their low activity. It has been found that a starter of low activity will result if such powders are used for the propagation of starters.

*Method adopted for preparation of batch starter with spray, non-fat, dry milk solids.* The method developed for making the batch starter with non-fat dry solids was as follows: (a) Test both water and milk solids to be used in preparation of starter milk to determine their suitability for this purpose, using the methods outlined above. (b) For batch starter preparation measure the water into the starter can with a measuring stick or other suitable device. (c) Heat water to 26.7° C. and regulate steam so that the temperature does not rise higher than 29.4° C. (d) A detachable two bladed paddle was attached to the paddle shaft just slightly above the water line to facilitate the dissolving of the powder. The paddles were made so that they would sweep the water downward. (e) Add enough high grade, spray, non-fat, dry milk solids to provide a 10 per cent concentration. About 15 min. usually were required to dissolve the powder. (f) Remove the upper paddles, pasteurize the contents at 87.8° C. for 1 hr., and cool to 21.1° C. (g) Inoculate the reconstituted milk with an active starter culture. Slightly more culture (usually 4 to 6 oz. per 80 lb. of milk) was required than when whole milk was used. The rate of inoculation should be governed so that the acidity does not reach more than 0.95 per cent at the end of the incubation period of 15 hr. The temperature of incubation is a very important factor. It should be 21.1 to 22.2° C. for the entire period. (h) After incubation, cool to at least 7.2° C., if the starter is not to be used immediately. (i) Regulate rate of inoculation of the starter in the cheese milk according to the activity of the starter. With an active starter 50 to 60 lb. to 10,000 lb. of milk is sufficient to

TABLE 2

*Activity factors of barrel lots of spray, non-fat, dry milk solids*

| Date test begun | Lot no. | Bbl. no. | Unknown milk | Known milk | Activity factors of successive serial transfers in: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Unknown milk | | | | Known milk | | | |
| | | | | | Start of trial | 1st transfer | 2nd transfer | 3rd transfer | Start of trial | 1st transfer | 2nd transfer | 3rd transfer |
| 10/11/46 | 10226 | 2 | 0.18 | 0.19 | 0.45 | 0.45 | 0.44 | 0.45 | 0.40 | 0.41 | 0.41 | 0.43 |
| 10/11/46 | KL887 | 18 | 0.19 | 0.19 | 0.43 | 0.45 | 0.44 | 0.44 | 0.40 | 0.41 | 0.41 | 0.43 |
| 11/ 4/46 | KL887 | 34 | 0.20 | 0.19 | 0.45 | 0.45 | 0.43 | 0.40 | 0.43 | 0.43 | 0.42 | 0.41 |
| 11/18/46 | 10156 | 42 | 0.17 | 0.19 | 0.33 | 0.31 | 0.31 | 0.30 | 0.40 | 0.42 | 0.41 | 0.43 |
| 11/18/46 | 10156 | 43 | 0.12 | 0.19 | 0.34 | 0.36 | 0.31 | 0.34 | 0.40 | 0.42 | 0.41 | 0.43 |

provide a milling acidity of 0.5 to 0.6 per cent in 4.5 hr. from setting to milling. (It must be kept in mind that high cooking temperatures, poor quality milk, toxic substances and bacteriophage will hinder the acid development in the cheese regardless of the activity of the batch starter.)

## SUMMARY

(a) Milk reconstituted from spray, non-fat, dry milk solids produced cultures and starters more constant in activity from day to day than did selected whole milk.

(b) Certain water supplies were found to be unsuitable for reconstituting milk for starter. Studies have shown that the water, if other than distilled, should be tested to determine whether or not it provides a reconstituted milk suitable for starter cultures.

(c) Spray, non-fat, dry milk solids varied in ability to provide a satisfactory culture medium for *S. lactis* starter cultures. Results showed that individual barrels of milk powder should be tested to determine their suitability for starter milk.

(d) Methods are outlined for testing suitability of water and non-fat dry milk solids for preparation of starter milk. A method also is given for preparation of reconstituted spray, non-fat, dry milk solids for batch starter.

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# THE RELATIONSHIPS AMONG CRACKED SOYBEANS FED, BARN TEMPERATURE AND THE DEGREE OF UNSATURATION OF MILK FAT[1, 2]

J. B. FRYE, JR.,[3] C. Y. CANNON,[4] AND E. W. BIRD[5]
*Iowa State College, Ames*

The results of a previous study (6) indicated a close relationship between external temperature changes and variations in the iodine value of the milk fat produced. That such a relationship may exist has been suggested by Dean and Hilditch (5) and Hilditch and Sleightholme (7). Likewise, Regan and Richardson (9) observed that when the room temperature was elevated above 80 or 85° F., an alteration occurred in the characteristics of the milk produced, which included, among other things, an increase in the unsaturated compounds of the milk fat (when the temperature was above 90° F.).

This study was conducted to secure additional information about the relationships of feeding cracked soybeans, barn temperature and the degree of unsaturation of milk fat.

## EXPERIMENTAL PROCEDURE

*Plan of experiment.* Twelve Holsteins and four Ayrshires were selected during a preliminary period (4 wk.) and divided into four similar lots. The placing of the cows into four equal groups and the allotment of the mixtures fed to each group were accomplished by random selection. Alfalfa hay was fed *ad libitum* and grain was fed at the rate of 1 lb. for each 3 lb. of milk produced. The animals were milked thrice daily.

Three experimental periods of 39, 74 and 56 days were employed. During the first experimental period groups 1 and 2 were fed rations (table 1) contain-

TABLE 1

*Concentrate mixtures used*

| Ingredients | Mixture A | Mixture B |
|---|---|---|
| | (*lb.*) | (*lb.*) |
| Cracked corn | 400 | 400 |
| Oats | 200 | 200 |
| Wheat bran | 300 | 300 |
| Linseed meal | 125 | ..... |
| Cracked soybeans | ..... | 125 |
| Bonemeal | 18 | 18 |
| Salt | 9 | 9 |

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[1] The data contained in this paper are from a thesis presented by the senior author to the faculty of the Graduate School, Iowa State College, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Journal paper no. J1661 of the Iowa Agricultural Experiment Station. Ames. Project 692.

[3] Present address: Louisiana State University, Baton Rouge.

[4] Dairy Husbandry subsection.

[5] Dairy Industry section.

ing linseed meal (mix A) and cracked soybeans (mix B), respectively. Then the grain mixtures were reversed and fed for a period of 74 days. During the last experimental period the animals were fed the same rations that they received during the first period.

Variations of the iodine values with chronological time during a previous study (6) indicated the advisability of carrying a control group of cows for each of the feeds being studied. In this study, groups 3 and 4 were designated as the control groups and were fed mixtures A and B, respectively, for the duration of the experiment without a change in feed.

*Cream and butter oil samples.* The cream and butter oil for chemical analyses were obtained and handled as described previously (6). The iodine value of the milk fat was determined according to the Hanus method (2). The thiocyanogen value of the fat was determined according to the method described by Jacobs (8), with the exception that the thiocyanogen solution was prepared by the method described by Arup (1). Acid numbers of the butter oil and pH determinations of the cream and butter serum provided a measure of possible changes in the quality of the cream and the fat. Acid numbers were determined by the method of Breazeale and Bird (3).

The high correlation found between mean external temperature and the iodine value of milk fat during a previous study (6) prompted the keeping of a daily record of the temperature changes in the barn in which the cows were housed during the entire experiment.

*Methods of obtaining cream samples for chemical analysis.* Data were obtained to determine whether a composite cream sample of 1 day's milking or a sample from one milking during the day should be taken for chemical analysis. Comparisons of the iodine and thiocyanogen values of the milk fat from each milking with those of the milk fat from composite sample for the same day are presented in table 2. These data indicate considerable variation among the iodine and thiocyanogen values of the fat from milking to milking. There is as much variation between milkings as exists from day to day (as determined by analysis of the composite samples). Temperature fluctuations may be one of the factors responsible for these variations.

The iodine and thiocyanogen values of the milk fat from the evening milk are in most cases higher than those for the morning and noon milkings. The iodine equivalent to the oleic acid content of the milk fat [iodine value—(iodine value—thiocyanogen value) $\times 2$] does not vary in this fashion as consistently as does the iodine value.

Table 3 includes the pH determinations made on the cream and butter serum and the acid numbers of the butter oil from each milking and from a composite sample for the same day. Although there is considerable variation among the acid values of the milk fat from milking to milking, differences among the pH values of the cream and butter serum from milking to milking are virtually within the experimental error of the method, except for the data on January 23. Nevertheless, the variation encountered in the iodine, thiocyanogen and acid

TABLE 2

*Iodine and thiocyanogen values and the grams iodine equivalent to the oleic acid in 100 g. milk fat from morning, noon and evening milk and from a composite milk sample for the same day*

| Date samples taken | Iodine value | | | | | Thiocyanogen value | | | | | Grams iodine/100 g. milk fat equivalent oleic acid | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.M. | Noon | P.M. | Av. | Comp. | A.M. | Noon | P.M. | Av. | Comp. | A.M. | Noon | P.M. | Av. | Comp. |
| Jan. 5 | 34.45 | 35.09 | 33.48 | 34.34 | 34.55 | 32.08 | 32.92 | 31.40 | 32.13 | 32.11 | 29.71 | 30.75 | 29.32 | 29.92 | 29.67 |
| 9 | 32.77 | 32.78 | 33.63 | 33.06 | 32.52 | 29.90 | 28.28 | 30.21 | 29.46 | 29.60 | 27.03 | 23.78 | 26.79 | 25.86 | 26.68 |
| 12 | 32.90 | 32.69 | 34.05 | 33.21 | 33.24 | 30.13 | 30.02 | 31.42 | 30.52 | 30.17 | 27.36 | 27.35 | 28.79 | 27.83 | 27.10 |
| 16 | 33.62 | 33.99 | 35.36 | 34.32 | 34.86 | 30.37 | 30.78 | 32.12 | 31.12 | 31.36 | 27.12 | 27.57 | 29.06 | 27.91 | 27.86 |
| 19 | 31.84 | 31.89 | 32.00 | 31.91 | 31.93 | 29.08 | 29.51 | 28.87 | 29.15 | 29.40 | 26.32 | 27.13 | 25.74 | 26.39 | 26.87 |
| 23 | 33.33 | 34.59 | 35.12 | 34.34 | 34.45 | 31.39 | 31.82 | 32.82 | 32.01 | 32.37 | 29.42 | 29.05 | 30.52 | 29.66 | 30.29 |
| Av. | 33.15 | 33.50 | 33.94 | 33.53 | 33.59 | 30.49 | 30.56 | 31.16 | 30.73 | 30.84 | 27.83 | 27.61 | 28.37 | 27.93 | 28.08 |

TABLE 3

*Acid numbers of butter oil and the pH values of cream and butter serum from morning, noon and evening milk and from a composite milk sample for the same day*

| Date samples taken | Acid number of butter oil (ml. 0.1N KOH/10 g. fat) | | | | | pH of cream | | | | | pH of butter serum | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A.M. | Noon | P.M. | Av. | Comp. | A.M. | Noon | P.M. | Av. | Comp. | A.M. | Noon | P.M. | Av. | Comp. |
| Jan. 5 | 0.358 | 0.536 | 0.702 | 0.532 | 0.420 | 6.85 | 6.84 | 6.87 | 6.85 | 6.87 | 7.05 | 6.99 | 7.09 | 7.04 | 6.99 |
| 9 | 0.344 | 0.831 | 0.635 | 0.603 | 0.697 | 6.78 | 6.75 | 6.78 | 6.77 | 6.81 | 6.84 | 6.80 | 6.90 | 6.84 | 7.15 |
| 12 | 0.447 | 0.669 | 0.447 | 0.521 | 0.559 | 6.82 | 6.83 | 6.84 | 6.83 | 6.79 | 7.12 | 7.10 | 7.09 | 7.10 | 6.94 |
| 16 | 0.466 | 0.448 | 0.511 | 0.475 | 0.349 | 6.83 | 6.80 | 6.81 | 6.81 | 6.81 | 6.94 | 6.89 | 6.93 | 6.92 | 6.87 |
| 19 | 0.332 | 0.529 | 0.412 | 0.424 | 0.435 | 6.84 | 6.89 | 6.87 | 6.86 | 6.87 | 6.96 | 7.04 | 7.04 | 7.01 | 6.92 |
| 23 | 0.340 | 0.560 | 0.609 | 0.503 | 0.520 | 7.29 | 7.07 | 6.78 | 7.04 | 6.85 | 7.00 | 6.76 | 6.80 | 6.85 | 6.86 |
| 29 | 0.269 | 0.215 | 0.233 | 0.239 | 0.296 | 6.73 | 6.67 | 6.71 | 6.70 | 6.71 | 6.92 | 6.99 | 6.92 | 6.94 | 6.86 |
| Av. | 0.365 | 0.541 | 0.507 | 0.471 | 0.468 | 6.88 | 6.84 | 6.81 | 6.84 | 6.82 | 6.98 | 6.94 | 6.98 | 6.96 | 6.94 |

values of the milk fat from milking to milking indicate that daily composite cream samples should be taken.

As a check on the deterioration of the cream from the time it was separated until churned, pH determinations on the cream and butter serum were made periodically. These data (not shown) indicate that in-so-far as pH values and titratable acidities are criteria, there was no change in the cream or butter during the interval between churning and (later) rendering to furnish fat for the iodine and thiocyanogen values.

*Chemical constants of milk fat—effect of temperature.* The iodine and thio-

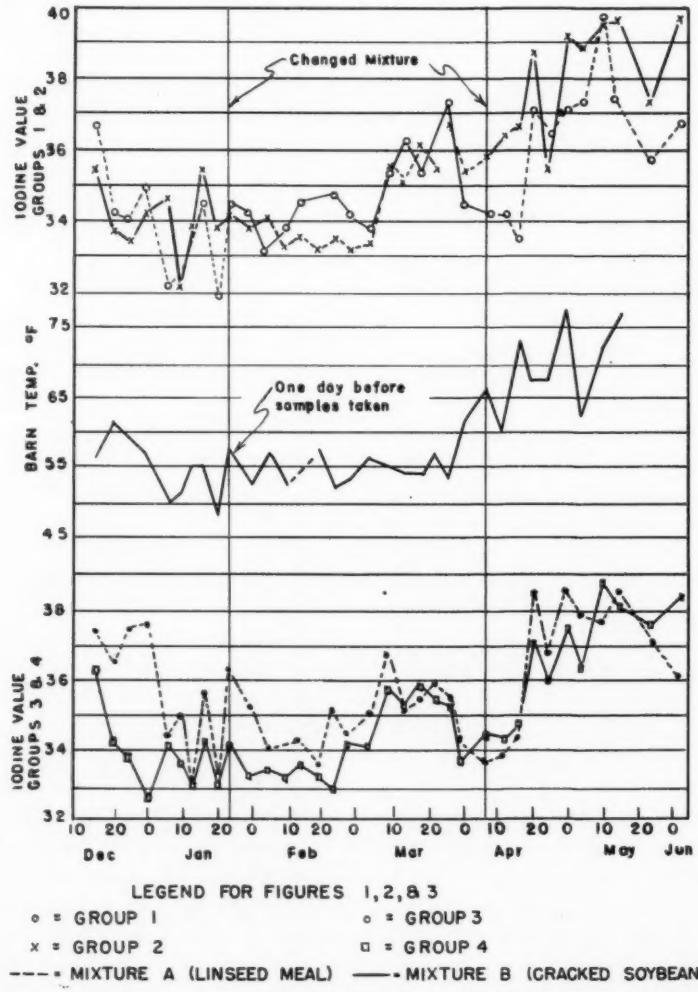


FIG. 1. Variation in iodine value and barn temperature with chronological time.

cyanogen values were employed as indices of the effect of feeds on changing milk fat composition. Previous work (6) indicated that temperature changes may play an important role in the variations of the iodine value of milk fat. A closer relationship existed between the iodine value of milk fat and the mean external temperature recorded 1 day before the samples were taken than that recorded on the same day or 2 days before. This relationship also was evident in this study.

Highly significant correlations were found between the iodine values of the milk fat and the mean barn temperatures (table 4) that were recorded the same day, 1 day before, and 2 days before the samples were taken. As the temperature increased, the iodine values tended to increase and vice versa (fig. 1). However, a closer correlation existed between the temperature recorded 1 day

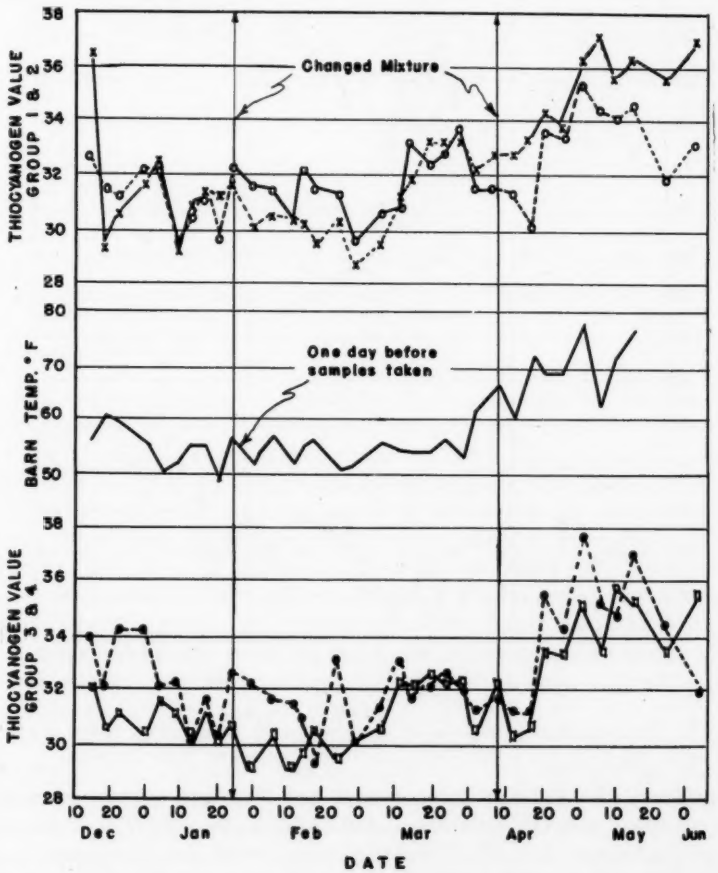


FIG. 2. Variation in thiocyanigen value and temperature with chronological time. (See fig. 1 for legends.)

before the samples were taken and the iodine value than that recorded on the same day or 2 days before. A closer correlation also existed between the temperature recorded the same day and the iodine value than that recorded 2 days before the samples were taken. The trends of the thiocyanogen values and their relationship to temperature changes (fig. 2) were similar in most respects to those of the iodine values. Generally, what was said about iodine values and the factors that influence them also may apply to the thiocyanogen values.

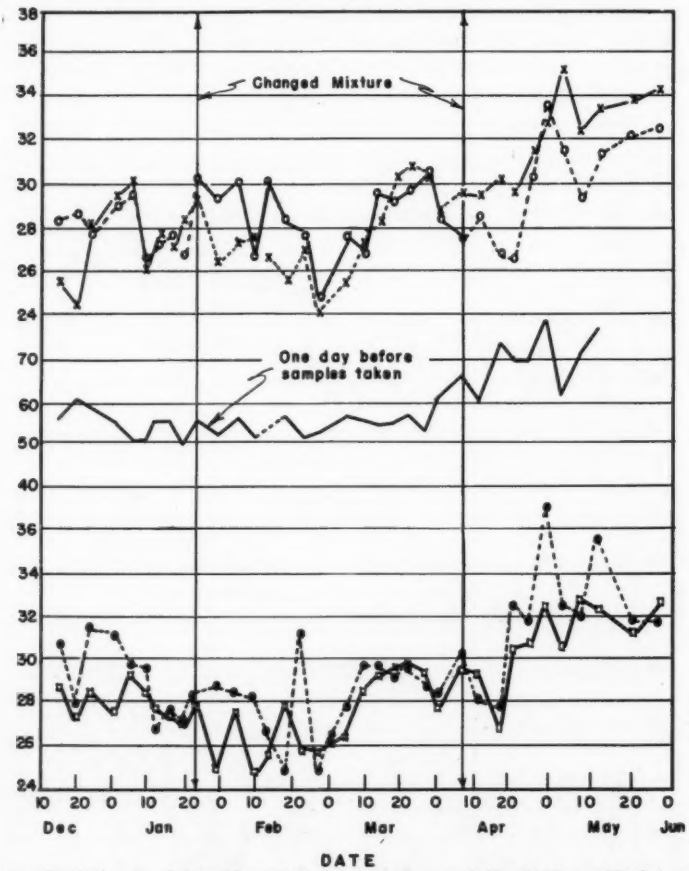


FIG. 3. Variation in oleic acid content (expressed as g. iodine/100 g. milk fat equivalent to oleic acid) and temperature with chronological time. (See fig. 1 for legends.)

The trends of the iodine, thiocyanogen and oleic acid (expressed as grams iodine per 100 g. milk fat equivalent to oleic acid) curves (fig. 1, 2, 3) are almost identical for each group. This is particularly true of the thiocyanogen curves of groups 3 and 4 (controls) (fig. 2). Since iodine and thiocyanogen are considered to add quantitatively to the single ethylenic linkage of oleic acid and

since there is more oleic acid than any of the other unsaturated fatty acids in milk fat, one would expect variations in the oleic acid content of milk fat to result in similar variations of the iodine and the thiocyanogen values. A significant correlation coefficient was found between the temperature recorded 1 day before the samples were taken and the oleic acid content of the milk fat (table 4).

TABLE 4

*Correlation coefficients between mean barn temperature recorded at various intervals and certain milk fat constants*

| Temperature[a] | Correlation coefficient | | | | |
|---|---|---|---|---|---|
| | Outcome group | Iodine value | Thiocyanogen value | Oleic acid[b] | Linoleic acid[c] |
| Same d. | 1 | 0.3526* | 0.4488** | 0.2942 | −0.1101 |
| 1 d. before | 1 | 0.5103** | 0.5593* | 0.4022* | 0.0119 |
| 2 d. before | 1 | 0.3046 | 0.3653* | 0.3058 | −0.0468 |
| Same d. | 2 | 0.6076** | 0.5199** | 0.3879* | 0.1430 |
| 1 d. before | 2 | 0.7628** | 0.6261** | 0.5725** | 0.0436 |
| 2 d. before | 2 | 0.5522** | 0.4945** | 0.4709** | 0.0580 |
| Same d. | 3 | 0.4573** | 0.5408** | 0.5290** | −0.3912* |
| 1 d. before | 3 | 0.5358** | 0.6139** | 0.6006** | −0.4030* |
| 2 d. before | 3 | 0.2756 | 0.3220 | 0.3307 | −0.2410 |
| Same d. | 4 | 0.5306** | 0.8939** | 0.4391* | −0.0357 |
| 1 d. before | 4 | 0.6843** | 0.7128** | 0.6858** | −0.2543 |
| 2 d. before | 4 | 0.4571** | 0.4858** | 0.5027* | −0.2656 |

[a] Temperature recorded at various intervals with relationship to the time the cream samples were taken.
[b] Grams iodine calculated as equivalent to oleic acid in 100 g. milk fat.
[c] Grams iodine calculated as equivalent to linoleic acid in 100 g. milk fat.
* Significant at 5% level.
** Significant at 1% level.

These data indicate that the changes in iodine value were largely dependent on the changes in the oleic acid content of the milk fat.

The high correlation between temperature changes and changes in the chemical composition of milk fat support the statement made previously in this paper that temperature fluctuations may be one of the factors responsible for daily variations of fat constants, even though the cows were fed the same feed.

*Effect of feed.* Results obtained in a previous study (6) indicated that the maximum effect of feed on the changes in milk fat composition, as measured by the iodine value, may be reached in approximately 15 days after a change in feed. However, the data obtained in this experiment (fig. 1) indicate that the time necessary for the feeds to produce their maximum effect on milk fat composition is not clear, except after the first change of feeds.

Following the change of rations on December 15, the differences between the iodine value curves of groups 3 and 4 (controls) became greater than did those between the curves of groups 1 and 2 (rations alternated). These differences decreased in magnitude and seemed to stabilize themselves fairly well in approximately 20 days. Concurrent with this stabilization period was a sharp drop in temperature and likewise a downward trend of the iodine value curves of all groups. In support of these findings, Dean and Hilditch (5) observed that

changes in iodine values were completed within 2 or 3 wk. after cows had gone on pasture in the spring. During the period December 15 to January 23, temperature and iodine value fluctuations were erratic. After this it was not possible to determine when the full effect of the feeds on milk fat composition had been reached.

The differences between the curves of groups 3 and 4 were smaller and more consistent throughout the experiment than were those between the curves of groups 1 and 2. This might be interpreted as indicating that the cows in groups 3 and 4 adjusted to the feeds so that the magnitude of the iodine values was about the same regardless of the feed. The recurrent crossing and the inconsistent differences of the curves of groups 1 and 2 as compared with those of groups 3 and 4 might indicate that the fat metabolism of the cows was being disturbed by the changes in feed.

*Iodine value and hardness of butterfat.* Coulter and Hill (4) found that the hardness of butter made under uniform conditions is closely dependent upon the hardness of butterfat. They also obtained a highly significant correlation between the hardness of butterfat and the iodine number.

In this study the iodine values of the milk fat from the animals (group 3) fed the linseed meal ration generally were higher than were those (group 4) fed the soybean ration. Moreover, an analysis of variance revealed that the differences, which were one unit or less in most cases, were significant ($F = -4.19$). However, it is doubtful whether a difference of one unit in iodine value would greatly affect the commercial processing of butter unless the iodine value is approximately 33 units (10). There was no significant difference between the iodine value of the milk fat from groups 1 and 2. Averages (35.1 and 35.3 for groups 1 and 2, respectively) for the entire experiment indicate that the differences between the iodine values were balanced during the experiment. However, the authors wish to point out that the differences at certain periods probably were great enough to produce varying effects on the quality of butter. For example, the differences in the iodine values of groups 1 and 2 from April 7 to 15, and from April 25 to June 1 (fig. 1) indicate that one could expect a difference in the body of butter produced by cows fed cracked soybeans or linseed meal at the same rates as were fed in this experiment. Although the iodine values of milk fats from cows fed either of these two feeds may be practically the same (especially over a long feeding period) at certain times, they also may be quite different at various temperature levels or at different stages of lactation or gestation. The feeding program used for the cows of groups 1 and 2 would not normally be followed by a dairy farmer, whereas that used for groups 3 and 4 would be more generally practiced.

## SUMMARY

By randomization four similar lots of four cows each were subdivided into four outcome groups and fed similar rations, except for the protein supplements (linseed meal and cracked soybeans). Two groups of cows were fed rations by the double reversal method, while a control group was fed each of the experi-

mental rations continuously throughout the experiment. Iodine, thiocyanogen and acid values of the milk fat and the pH of the cream and butter serum were determined. Barn temperatures were recorded daily.

In studying changes in fat composition, it seems advisable to utilize fat samples representative of one full day's milk yield, rather than samples obtained from individual milkings.

Highly significant correlations existed between the mean barn temperature (recorded the same day, 1 day before, and 2 days before the cream samples were taken) and the iodine and thiocyanogen values of the milk fat. The temperature recorded 1 day before the samples were taken had a closer correlation to changes in fat composition than that recorded the same day or 2 days before they were taken. The authors wish to point out that the correlation between the iodine and thiocyanogen values of the milk fat and temperature existed with cows which were from 1 to 2.5 mo. along in lactation when the experiment started (late November). It might be that had the animals freshened during November, February or April a different picture would have resulted (relative to apparent temperature effects) because of the lactation cycle.

The maximum effect of changing feeds on milk fat composition, as measured by the iodine and thiocyanogen values, appeared to have been reached in approximately 20 days after the first change of feeds. Apparently such factors as temperature changes and progress of the lactation and gestation periods interfered with fat metabolism so that it was impossible to determine when the full effect of the feeds had been reached following succeeding changes. Unless certain uncontrolled factors complicate the picture it appears from this study and a previous one (6) that 15 to 20 days may be required for a feed to exert its full effect on milk fat composition. This is in agreement with the work of Dean and Hilditch (5).

When the cows were fed either linseed meal or cracked soybeans as 11.1 per cent of the concentrate mixture over a long feeding period, they appeared to adjust themselves to the rations so that the iodine values of their milk fat were of about the same magnitude and their differences fairly consistent. On the other hand, when these feeds were fed for short feeding periods and then changed, the fat metabolism of the cows seemed to be disturbed so that the differences between the iodine values of the milk fat were rather inconsistent.

The changes in iodine values were largely dependent on the changes in the oleic acid content of the milk fat.

Non-significant differences were found between the iodine values of the milk fat from cows alternated from the linseed meal ration to the soybean ration and vice versa. However, the differences at certain periods were probably great enough to produce different effects on the quality of butter. A significant difference was found between the iodine values of the milk fats from cows fed linseed meal and cracked soybeans continuously at the rate of 11.1 per cent of the concentrate mixture. The iodine values of the milk fat from cows fed the linseed meal ration generally were higher throughout the experiment than were those of the milk fat from cows fed the cracked soybean ration. Although the

iodine values of the milk fat from cows fed either of these two concentrates may be practically the same (especially over a long feeding period) at certain times, they also may be quite different at various temperature levels or at different stages of lactation or gestation.

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The authors are grateful to N. E. Fabricius, currently with the Ladysmith Milk Producer's Cooperative, Ladysmith, Wisconsin, and to B. W. Hammer for valuable help given in scoring the milk and cream and to G. W. Snedecor and W. G. Cochran for advice in the statistical analysis of the data.

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# THE RELATION BETWEEN THE DEGREE OF SOLIDIFICATION OF FAT IN CREAM AND ITS CHURNING TIME. II. THE PHYSICAL DISTRIBUTION OF THE LIQUID–SOLID PHASES WITHIN THE GLOBULE

J. ROBERT BRUNNER

*Michigan Agricultural Experiment Station*[1]

AND

E. L. JACK

*Division of Dairy Industry, University of California*

The influence of variations in churning temperature of cream upon its churning time is common knowledge. As the temperature of cream is lowered, within the churning limits, the churning time is increased. It is believed that some of the variations in churning result from changes in the degree of solidification of the fat. This supposition is well supported by observation. Undoubtedly, lowering the temperature of the cream results in greater crystallization of the fat. The fact that satisfactory churning occurs when the temperature of the cream is within rather restricted limits suggests that, at churning temperatures, the degree of solidification in the fat is quite specific.

The studies of van Dam and Burgers (4), van Dam (3), Rishoi and Sharp (9), Jack (6), Coulter and Combs (2) and others offer evidence that milk fat exists in a state of partial solidification, both in the cream at the churning temperature and in the churned butter. The extent of fat solidification is somewhat controllable by process temperature manipulation. Haglund *et al.* (5), Richardson and Abbott (8), Coulter and Combs (2), Wilster *et al.* (11) and others have emphasized that such measures often are necessary to produce a butter with desirable body and texture when made from milk fats that are abnormally hard or soft.

The purpose of this study was to correlate the degree of solidification in the fat with the churning time of the cream and to determine to what extent processing procedures affect the solidification of fat in cream prepared for churning.

## EXPERIMENTAL METHODS

To determine the extent that processing procedures affect the degree of solidification in globular fat, the following experimental procedure was followed: One hundred pounds of freshly-separated cream testing 33 per cent fat was pasteurized at 66° C. for 30 min. One-third of this cream, designated as lot A, was cooled and held at 0° C. for 18 hr. prior to churning. A second portion of the cream, lot B, was cooled and held at 10° C. for 18 hr. The remaining portion of the cream, lot C, was held at 60° C. while 5-lb. quantities were removed, cooled in ice-water to the churning temperature and immediately churned in a tempera-

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ture-controlled, motor-driven Daisy churn of an agitator type. Likewise, after cream lots A and B had been held for the designated 18-hr. holding period, 5-lb. portions were removed, adjusted to the churning temperature and immediately churned. A calorimetric technique, suitable for measuring the percentage of solid fat in cream at the churning temperature, as used previously by Jack and Brunner (7), was employed to determine the degree of solidification. Churning temperatures were selected to cover the churning range.

## RESULTS

When churning times of cream are plotted as ordinates against percentage of solidified fat therein (degree of solidification) as abscissa, curves are obtained which, in the desirable churning range of 35 to 55 min., are logarithmic (figure 1).

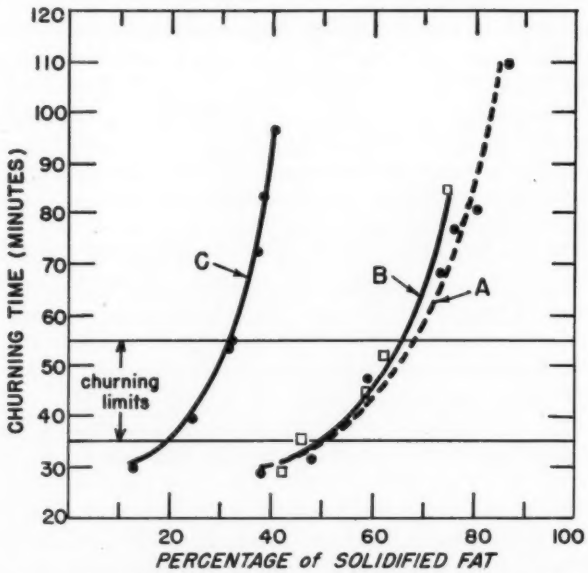


FIG. 1. The relation between the degree of solidification of milkfat at the churning temperature and the churning time of the cream (33.0% fat). Curve A—Cream held at 0° C. for 18 hr. and warmed to the churning temperature. Curve B—Cream held at 10° C. for 18 hr. and warmed to the churning temperature. Curve C—Cream pasteurized at 66° C. and cooled to the churning temperature.

Below these time limits, churnings are incomplete and accompanied by excessive fat losses in the buttermilk, while those above proceed with difficulty, if at all. Within the desirable churning range, creams from lot C contained between 20 and 31 per cent solidified fat, while creams from lots A and B contained between 48 and 68 per cent of the fat in the solid state. Cream from lot A contained slightly more crystallized fat than cream from lot B, which would be expected, since more fat should be solidified at 0° C. than at 10° C. The more fat that is

in a solid state, the greater is the time required for churning; however, within the three lots studied, the churning time depends more upon the cooling procedure used than on the percentage of solidified fat. For example, as shown in figure 1, cream from lot A, held at 0° C. for 18 hr. prior to churning, and lot C, churned immediately after cooling, containing approximately 40 per cent solidified fat churned in 30 and nearly 100 min., respectively.

The fact that a wide variation exists between the percentage of solid fat in churnings from lots A and B and lot C indicates that the degree of solidification

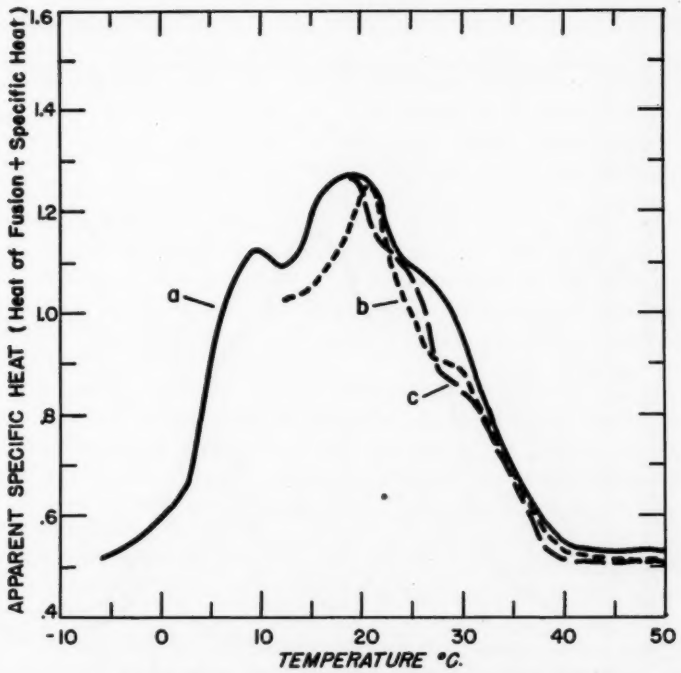


FIG. 2. Apparent specific heat values over the temperature range studied of fat in cream held at 0° C. for 18 hr. and warmed to the churning temperature. Curve a—Control, cream held at 0° C. for 18 hr. Curve b—Cream churned at 11.10° C. Curve c—Cream churned at 13.90° C.

occurring at the time of churning is not nearly as significant in the time required for churning as is the probable position of the solidified fat in the globule. Some insight as to the general location of this solidified globular fat might be obtained (a) by applying the principle of the second law of thermodynamics which states, essentially, that heat is conducted from an area of high heat level to an area of low heat level until a state of temperature equilibrium is reached and (b) from an inspection of the apparent specific heat curves of the fats involved, which, together with the work of van Dam (3) and Rishoi and Sharp (10), show that fat

exhibits a considerable time lag in attaining thermal equilibrium throughout the fat globule.

The globular fat in cream as prepared from lot A presumably is in a state of nearly complete solidification. When portions of this cream are warmed to the churning temperature, heat is transmitted to the continuous serum phase from which it is transferred to the fat phase. Due to the poor thermal conducting properties of fat, the heat transfer process proceeds inward through the fat at a relatively slow rate. This lag in attaining equilibrium probably results in the creation of a layer of partially liquid or softened fat on the outer surfaces of the fat globule. To support this assumption it is necessary to demonstrate that the

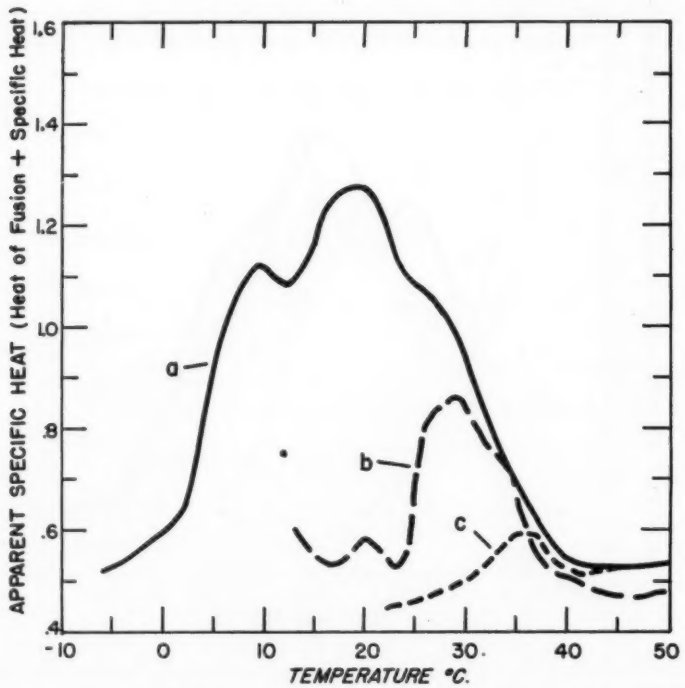


FIG. 3. Apparent specific heat values over the temperature range studied of fat in cream pasteurized at 66° C. and immediately cooled to the churning temperature. Curve a—Control, cream held at 0° C. for 18 hr. Curve b—Cream churned at 10.60° C. Curve c—Cream churned at 19.10° C.

remaining portion of solidified fat, supposedly located in the interior of the fat globule, has not been liquified in the warming process.

In figure 2, the apparent specific heat curves of fat from churnings *b* and *c*, cream processed as in lot A, are compared with a similar curve of fat in a state of nearly complete solidification (curve *a*). The partial curves *b* and *c* coincide fairly well, in the range covered, with that of the control, curve *a*. Since ap-

parent specific heat measurements are made by determining the amount of heat necessary to change the temperature of the material, this indicates that the fat being liquefied from the churning temperature upward to a state of complete liquefaction is fat in the original state of equilibrium. Therefore, the heat applied to the cream system in raising the temperature from 0° C. to the churning temperature probably was manifested in melting or softening outer layers of the globular fat, rather than being distributed evenly throughout the globule, since the residual solid fat is still in the state of equilibrium existing at 0° C.

Similar reasoning can be applied to creams prepared for churning as in lot C. When cooling cream from 60° C. to the churning temperature, heat is transferred from the serum to the cooling medium, then by a somewhat slower process from the fat globules to the serum. By this process, there probably is formed a layer of solid or liquid-solid fat surrounding the fat globules, leaving a large portion of the interior fat in a liquid state. In figure 3, the apparent specific heat curve *a*, like curve *a* in figure 2, is representative of globular fat in a state of nearly complete solidification, while curves *b* and *c* represent churning samples. At the time churning was started, immediately after the cream was cooled, and at the beginning of apparent specific heat determinations, very little of the fat had been solidified. However, after a period of 20 to 30 min. in the calorimeter, some crystallization of the fat occurred, as indicated by the maxima in curves *b* and *c*. As assumed in the preceding case, this change in physical state occurred principally in the fat globule surface layers.

The conjecture is made that the physical state of the globular fat on the surface layers is somewhat similar in creams that churn within desirable churning limits, regardless of the procedure used in preparing the creams for churning. The fat on the surface of the globule is neither totally liquid nor totally solid but in a soft semi-solid, sticky condition conducive to fat globule agglomeration. The fact that more solidified fat is formed in one method of processing than in another has little influence upon the churning time, but is manifested more significantly in the completeness of churning, as well as in the body and texture of the final butter.

Richardson and Abbott (8) have indicated that cream cooled to churning temperature immediately after pasteurization should be churned at a lower temperature than cream that has been warmed to churning temperature. The data given in figure 4 show that cream samples prepared by cooling from the pasteurization temperature (lot C) required somewhat higher temperatures to effect satisfactory churnings in the same period of time than did creams warmed to the churning temperature (lots A and B). By cooling the cream with ice-water, it is possible that a layer of solidified fat was formed on the fat globule surface which interfered with fat coalescence beyond the effect of agitation, resulting in retarded churnings. This supposition is in harmony with the reasoning advanced in this report.

In attempting to analyze the results obtained from a churning study of this nature the possibility of alteration in crystalline form should not be neglected as a means of explaining some of the variations in the churning behavior of

cream. Clarkson and Malkin (1), employing X-ray diffraction technique and melting curves, were able to demonstrate in pure triglycerides three distinct polymorphic crystal formations depending upon the rate of solidification. Slow cooling results in the formation of the most stable form designated by them as beta and having the highest melting point. More rapid cooling results in the production of an intermediate alpha form, while extremely rapid chilling will produce the unstable gamma or "glass" form. Upon aging or the application of heat, the unstable monotropic gamma and alpha forms will transform irreversibly into the more stable beta form.

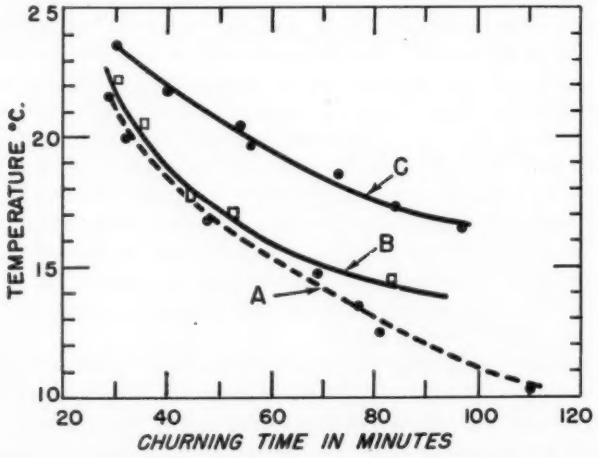


FIG. 4. The relation between the churning time of cream and the churning temperature. Curve A—Cream held at 0° C. for 18 hr. and warmed to the churning temperature. Curve B—Cream held at 10° C. for 18 hr. and warmed to the churning temperature. Curve C—Cream pasteurized at 66° C. and cooled to the churning temperature.

X-ray diffraction technique and melting curve studies, representing the best methods available for observing polymorphic transitions in triglycerides, when used to study the pure and simple triglycerides give only qualitative data; indeed, the difficulties encountered in trying to observe polymorphic transitions in a naturally occurring, mixed glyceride such as milk fat in the globular state would present a technical problem of some magnitude. Hence, certain facts obtainable from a study of the more simple glycerides are used to help explain phenomena occurring in the more complex natural fats.

The temperature history of cream samples churned from lots A and B indicate that for all practical purposes the fat is in a state of stable equilibrium (Malkin's beta crystals) at the end of the 18-hr. tempering period (Rishoi and Sharp, 10). Upon warming the cream, the added heat is expended in melting a portion of the solidified fat and raising the over-all temperature to the desired churning temperature. The possibility of reverse polymorphic transition is precluded, since the gamma alpha beta transitions are monotropic. On the other hand, when

cream samples from lot C are prepared for churning by relatively quick cooling from 60° C., the opportunity exists for the formation of the intermediate alpha and possibly the unstable "glass" types of crystallization. It is the authors' postulation, however, that if these polymorphic forms are crystallized their existence at the churning temperature would be relatively short-lived, since the transition of the unstable forms to the stable beta form is the normal transition pattern. Therefore, it is felt that the churning data obtained in this study are influenced primarily by the occurrence and distribution of solid and liquid fat in the globule as deduced from the specific heat curves shown herein. However, the possible role played by different polymorphic forms of crystalline milk fat should not be disregarded entirely, even though the data presented are not designed to demonstrate polymorphic transitions.

## SUMMARY

A calorimetric technique was used to determine the percentage of crystallized milk fat (degree of solidification) present in creams prepared for churning by the following methods:

Lot A. Cream was cooled and held at 0° C. for 18 hr. prior to churning at selected temperatures.

Lot B. Cream was cooled and held at 10° C. for 18 hr. prior to churning at selected temperatures.

Lot C. Cream was cooled in ice-water from 60° C. to the desired temperature and immediately churned.

The degree of solidification in creams from lots A and B, churning within 35 to 55 min., ranged between 48 and 68 per cent solidified fat. Cream portions from lot C that churned within the same time range contained between 20 and 31 per cent solidified fat.

Within any one lot of cream, the more solidified fat that is present at the churning temperature, the longer is the time required for churning.

The experimental results indicate that the churning time is not entirely dependent upon the actual degree of solidification in the fat, but, to a greater extent, upon the distribution of the liquid and solid fat phases on the globular fat surfaces.

Polymorphic transition of the crystallized fat has been considered as a possible explanation for the churning results obtained.

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# ELECTROPHORESIS OF MILK PROTEINS. I. SOME COMPARISONS OF SALT-ACID AND SALT-LYOPHILIZED WHEY FRACTIONS[1, 2]

WILLIAM G. STANLEY, A. C. ANDREWS AND C. H. WHITNAH
*Department of Chemistry, Kansas State College, Manhattan*

There are several known methods used for the isolation of whey proteins from milk. Harland and Ashworth (3) have outlined a salt-acid method which is perhaps one of the simplest and easiest. Their procedure involves the precipitation of casein by saturation of skim milk with NaCl followed by precipitation of the whey proteins upon addition of HCl to the resulting filtrate. Harland and Ashworth claimed that whey proteins prepared by this method were "relatively undenatured." However, it is possible that addition of HCl to the non-casein filtrate might change the characteristics of the original whey proteins. Such changes in these protein fractions have been suggested by Whitnah *et al.* (13) in their studies of the surface tension of milk. Differences between the electrophoretic patterns for Harland and Ashworth whey proteins and for those obtained from whey proteins isolated by other methods should show possible changes in the whey proteins due to the effect of the precipitating reagents. The usual procedure employed in the preparation of whey proteins for electrophoretic studies involves the precipitation of casein from skim milk by acid (Smith, 10) or rennet (Deutsch, 2), followed by the concentration of the casein-free proteins by lyophilization.[3]

Harland and Ashworth proteins and whey proteins prepared by a modified Harland and Ashworth procedure were used in the present study. In the latter method the whey proteins were concentrated by lyophilization of the non-casein filtrate, rather than by acid precipitation. Any differences between these two patterns should indicate an effect of acid precipitation on the whey proteins. Comparison of the electrophoretic components of each of these preparations with corresponding components in the preparations of Smith and Deutsch might indicate possible effects due to the salting out of the casein.

Harland and Ashworth concluded that their "non-protein filtrate" was completely free from whey proteins and that the remaining nitrogenous substances were not protein in character. In the salt-lyophilized method of the present study this filtrate was still present in the sample. Electrophoresis should prove the presence or absence of any whey proteins not precipitated or of any non-dialyzable, non-protein nitrogenous substances in this non-protein filtrate.

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[2] This paper contains part of the material presented by William G. Stanley in a thesis for the Master of Science Degree at Kansas State College. 1950.

[3] In this paper the word "lyophilize" indicates the removal by sublimation in a vacuum of some water from a frozen solution.

PROCEDURE

Pooled whole raw milk for this work was obtained from the Kansas State College dairy. The cream was separated from the skim milk with a Sharples super centrifuge. The skim milk was saturated with NaCl at 40° C. and allowed to stand overnight at this temperature. The casein was filtered off and discarded. The non-casein filtrate was divided into two parts, A and B (fig. 1). Part A was

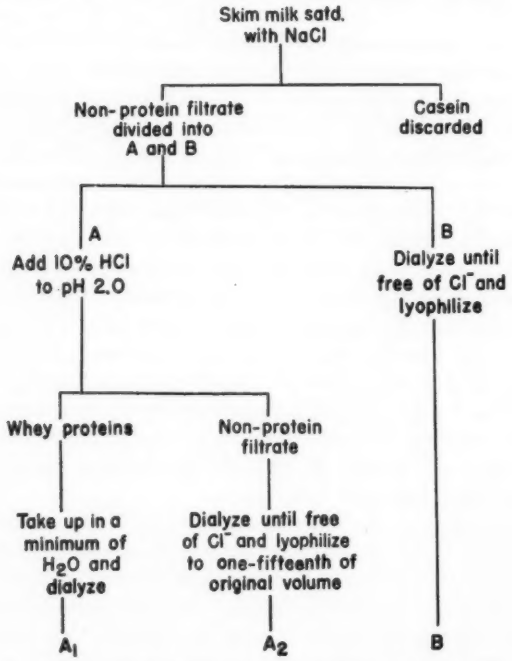
Skim milk satd. with NaCl
Non-protein filtrate divided into A and B
Casein discarded
A
Add 10% HCl to pH 2.0
B
Dialyze until free of Cl⁻ and lyophilize
Whey proteins
Non-protein filtrate
Take up in a minimum of $H_2O$ and dialyze
Dialyze until free of Cl⁻ and lyophilize to one-fifteenth of original volume
$A_1$
$A_2$
B

FIG. 1. Flow sheet for preparation of fractions of milk proteins.

adjusted to pH 2.0 by addition of 10 per cent HCl with rapid stirring and allowed to stand overnight at room temperature. This standing both at 40° C. and at room temperature was a definite part of the Harland and Ashworth procedure. The precipitated whey proteins were collected on a filter paper and transferred to a cellophane dialysis sack with a minimum of distilled water. Whey proteins prepared by this method were similar to the Harland and Ashworth proteins and designated as $A_1$ in figure 1. The non-protein filtrate from A was dialyzed against distilled water at 4° C. until free of chloride ion. The non-protein filtrate was then lyophilized to one-fifteenth of its original volume and designated as $A_2$. Part B was dialyzed against water at 4° C. until free of chloride ion and then lyophilized. This fraction was designated as B.

Fractions $A_1$ and B were diluted with a veronal-citrate buffer of 0.088 ionic strength and pH 7.6 until the protein concentration was approximately 0.85 per

cent and dialyzed in 2-l. bottles at 4° C. for 72 hr. against three changes of buffer solution. Sample $A_2$ was dialyzed without dilution in the same manner. The final buffer solutions, against which the samples were dialyzed, were used as the solvents in the electrophoresis cells to form the boundaries for migration. The veronal-citrate buffer employed was prepared by bringing a solution of 206.18 g. of sodium barbital and 45 g. of sodium citrate dihydrate to pH 7.6 by the addition of citric acid and diluting to 20 l. with distilled water.

Electrophoresis was carried out for 7,680 sec. at 0.5° C. using a modified Tiselius apparatus manufactured by the Klett Manufacturing Co., New York City (1, 4, 5, 6). A constant current of 20 ma. for the electrophoresis was supplied by the current regulator as designed by McColloch (7). Patterns were recorded on Kodak Metallographic plates using the Schlieren scanning method (5).

To facilitate the measurement of peak areas, the patterns were enlarged approximately four times by projecting them on a sheet of paper. The enlarged images were traced on the paper and component areas measured with a planimeter. In determining the area due to a given component, a more or less arbitrary division of a pattern had to be made due to the overlapping of the peaks. Following the procedure of Tiselius and Kabat (12), an ordinate was drawn from the lowest point between two adjacent peaks, while use of the method of Svedberg and Pedersen (11) involved division of the pattern into a series of symmetrical curves. The results utilizing both methods are recorded in table 1.

TABLE 1

*Areas of electrophoresis components from whey proteins*

| Component | Ascending boundaries | | | | Descending boundaries | |
|---|---|---|---|---|---|---|
| | $A_1$ Salt-acid | | B Salt-lyophilized | | Smith | Deutsch |
| | Tiselius & Kabat | Svedberg & Pedersen | Tiselius & Kabat | Svedberg & Pedersen | * | – |
| | % of total area | | | | | |
| 1 | 3.0 ± 0.1** | 2.7 ± 0.1 | 7.4 ± 0.2 | 7.2 ± 0.3 | 6 | 10.2 |
| 2 | 7.1 ± 0.1 | 6.8 ± 0.3 | 4.1 ± 0.1 | 3.4 ± 0.4 | 4 | |
| 4 | 15.8 ± 0.1 | 12.9 ± 0.1 | 17.9 ± 0.2 | 16.9 ± 0.4 | 21 | 18.0 |
| 4 | 7.4 ± 0.4 | 7.4 ± 0.3 | 8.4 ± 0.3 | 8.0 ± 0.4 | 13 | 20.2 |
| 5 | 61.8 ± 0.6 | 66.0 ± 0.4 | 56.0 ± 0.3 | 58.7 ± 0.7 | 51 | 48.3 |
| 6 | 5.0 ± 0.3 | 4.2 ± 0.2 | 6.1 ± 0.2 | 5.0 ± 0.3 | 5 | 3.3 |

* Interpolated from values for 0.51 and 1.23% protein to 0.85% protein.
** Standard error of mean, calculated from measurements on 5 projections.

Patterns obtained for the ascending boundaries were used. The electrophoresis time at which best measurements of both patterns could be made was 5,880 sec.

A micro-Kjeldahl method as described by Niederl and Niederl (8) was used to determine the nitrogen concentration of each of the samples. Protein concentrations were calculated using a conversion factor of 6.38.

## RESULTS AND DISCUSSION

The electrophoretic patterns of whey proteins prepared by either method contained six electrophoretically distinguishable peaks in relatively the same positions (fig. 2). The patterns showed no qualitative differences in the nature of

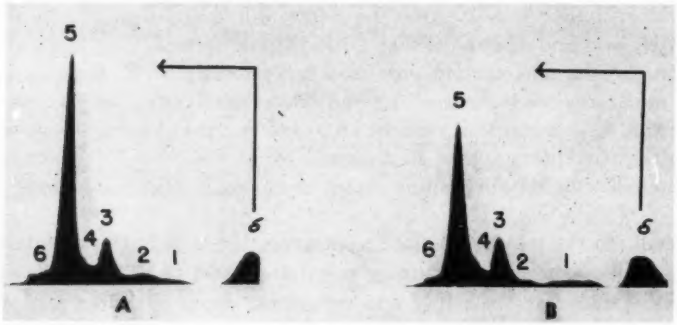


FIG. 2. Electrophoresis of whey proteins ascending boundaries. A. Salt-acid 5,880 sec., 0.87% protein; B. Salt-lyophilized 5,880 sec., 0.815% protein.

whey proteins prepared by either method. There were, however, quantitative differences, as shown in table 1. The concentration of component 1 by the salt-acid method was less than half the salt-lyophilized value, while the salt-acid method for component 2 gave a value nearly double that from the salt-lyophilized method. These changes were far greater than would be allowed either by the standard errors shown in table 1, or by any uncertainties of the relation between concentration and area. Smith (10) identified component 1 as euglobulin and component 2 as pseudoglobulin.

Longsworth (4), working with human blood plasma, found that the two methods for measuring component areas differed by as much as 33 per cent with an average difference of 15 per cent. From values in table 1 corresponding differences in this study were 18 and 7.5 per cent, respectively.

For components 1 to 5 the data from salt-lyophilized proteins agreed with Smith's data better than the data from salt-acid proteins. This was also true of Deutsch's data except that he did not divide components 1 and 2. Only for component 6 did the data from salt-acid proteins agree somewhat better with the data of both Smith and Deutsch.

Smith used a veronal buffer of ionic strength 0.1 at pH 8.6. Deutsch used a buffer similar to the present study, except at pH 8.6 rather than 7.6. The protein concentration of his electrophoresis solution was not given. Such differences in method may account for some of the differences in peak concentrations.

According to Rowland (9) and confirmed by Harland and Ashworth, 5.64 per cent of the total nitrogen in milk is present in the form of non-protein nitrogen. In preparation of whey proteins by the salt-acid method, all of this was present in the non-protein filtrate; however, by the salt-lyophilized method, only the casein was discarded, the non-casein filtrate being lyophilized in making sam-

ple B. Thus, any non-dialyzable nitrogenous substance in the non-protein filtrate would still be present in sample B. It first was thought the increase in concentration of the euglobulin by the salt-lyophilized method could be due in part to such foreign substances. However, sample $A_2$, as prepared for electrophoresis, produced only a minute pattern (fig. 3). This finding has been confirmed on later

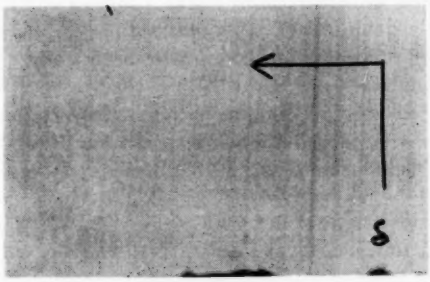

FIG. 3. Electrophoretic pattern of non-protein filtrate from salt-acid whey. 5,400 sec., $N \times 6.38/100 = 0.035$.

samples concentrated to as little as 1/75th the volume of the original non-protein filtrate.

The original milk may be assumed to have contained 0.08 per cent nitrogen from whey proteins and 0.03 per cent non-protein nitrogen. These would correspond, respectively, to 0.51 per cent protein and 0.19 per cent protein equivalent ($N \times 6.38$). If none of this non-protein nitrogen was dialyzable, the solution $A_2$ when concentrated to one-fifteenth its original volume should have contained 0.19 per cent of 15 or 2.85 per cent protein equivalent. Analysis showed 0.035 which is only 1.2 per cent of the expected 2.85.

The electrophoretic pattern to be expected from such an assumed solution of 2.85 per cent protein equivalent should have an area nearly three and five-tenths times the area of the salt lyophylized protein (B, fig. 2) containing 0.82 per cent protein. The observed pattern (fig. 3) was negligibly small.

The small observed peaks could have been due either to a small amount of non-dialyzable, non-protein, nitrogenous material or to a small amount of whey proteins that was not filtered from the non-protein filtrate. It must be emphasized that these determinations were only approximate and preliminary, as no quantitative measurements of these non-protein substances have been attempted by electrophoresis. It is possible then, from this evidence, that the apparent concentration change of the 1 and 2 components could have been due to some chemical action of the HCl upon the immune lactoglobulins, resulting in a change of their electropohoretic behavior.

## SUMMARY

1. Electrophoretic patterns were obtained for whey proteins. These proteins were prepared by two different procedures, namely, salt-acid and salt-lyophilized.

2. Six electrophoretically distinguishable components were split off by electrophoresis of these whey proteins.

3. No qualitative difference in whey proteins prepared by these methods was recognized.

4. The electrophoretic concentration of euglobulin by the salt-acid method was less than half the salt-lyophilized value, while for pseudoglobulin the salt-acid value was nearly double the salt-lyophilized.

5. The change of concentration could have been due to action of HCl upon the immune lactoglobulins.

6. Relative percentages of component areas from salt-lyophilized proteins agreed qualitatively with those obtained by Smith and only for one component did areas from salt-acid proteins agree better with the data of Smith and of Deutsch.

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# STALE-FLAVOR COMPONENTS IN DRIED WHOLE MILK. III. THE STEAM DISTILLATION OF STALE-FLAVOR COMPONENTS FROM STALE BUTTER OIL

R. McL. WHITNEY, KATHERINE PAULSON AND P. H. TRACY
*Department of Food Technology, University of Illinois*

In previous papers in this series (1, 2), it was demonstrated that the stale-flavor component which develops in dried whole milk upon storage could be removed from the milk powder with the butter oil. Since this component was believed to be volatile, preliminary tests were made with a conventional steam distillation apparatus which indicated that the stale-flavor component could be separated from stale butter oil by this technique. This study was undertaken to verify these observations and to develop the most satisfactory procedure.

## EXPERIMENTAL

*Preparation of the stale butter oil.* Whole milk from the University herd was condensed, dried without previous homogenization in a pilot-sized experimental spray drier and stored under varying conditions in order to have a continuous supply of stale, dried whole milk. Stale butter oil was prepared as needed from the stale milk powder by two of the extraction procedures described in a previous paper (2). In some cases, the powder was hydrated to approximately 8 per cent moisture by the dynamic method of Wilson (3) before extracting with petroleum ether, and, in the rest, the alcohol pretreatment was used. After extraction, the solvent was removed from the stale butter oil by the special technique previously reported (2). When not used immediately, the stale butter oil was stored at -26° C.

*Steam-distillation apparatus.* While some changes were made in the apparatus throughout this study, it remained essentially a typical assembly for steam distillation under controlled pressures. The following description applies to its final form. Steam was generated in a 2-l. round-bottom flask immersed in a constant-temperature water bath. This boiler was equipped with a vacuum or pressure-release valve placed in the steam line to prevent either an explosion or the drawing back of oil from the distilling flask into the boiler. Steam from the boiler was bubbled through the melted butter oil in a 1-l. Claisen distilling flask. A thermometer, standardized against a Bureau of Standards thermometer, and a closed-end type mercury manometer were connected at the outlet of the flask to measure the temperature and pressure of the distillate at this point. The distillate then was passed through a water-cooled condenser and allowed to escape beneath the surface of the receiving liquid in a graduated cylinder maintained at as low a temperature as was practical for the particular receiving liquid. The reduced pressure in the system was obtained by a high-vacuum pump connected to the receiver through a manostat. To aid in adjusting the manostat, another closed-end type mercury manometer was inserted between it

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and the receiver. The pump was protected against moisture by means of a drying tube filled with $CaCl_2$ with "Tell-Tale" desiccant (silica gel) as an indicator of saturation.

*Experiments.* In order that the development of the final procedure and the interpretation of the results may be followed more easily, the results of the key

TABLE 1

*The effect of steam distillation of stale butter oil upon the distribution of stale-flavor component*

| Expt. no. | Major difference from previous experiment[a] | Threshold value of: Original stale butter oil[b] | Pretreated butter oil[b] | Residual stale butter oil[b] | Fresh butter oil plus distillate[b] | Petroleum ether-soluble volatile fraction[b] |
|---|---|---|---|---|---|---|
| | | (%) | (%) | (%) | (%) | (*p.p.m.*) |
| 1 | ..... | (6)1.8 ± 0.3 | ..... | (4)2.5 ± 0.6 | (6)2.0 ± 0.9 | ..... |
| 2 | Fresh butter oil distilled | (2) > 4.0 | ..... | (2) > 4.0 | (2) > 4.0 | ..... |
| 3 | Distillation of stale butter oil under reduced pressure | (6)2.1 ± 0.9 | ..... | (6)1.8 ± 0.1 | (8)1.8 ± 1.4 | ..... |
| 4 | Distilled $H_2O$ as receiving liquid | (6)1.8 ± 0.4 | ..... | (12)1.8 ± 0.4 | (2) > 4.0<br>(6)2.0 ± 1.3 | ..... |
| 5 | Distillation at atmospheric pressure | (6)1.8 ± 0.4 | ..... | (10)1.2 ± 0.6 | (2) > 4.0<br>(10)2.6 ± 1.2 | ..... |
| 6 | Pretreatment and distillation at reduced pressures | (6)1.5 ± 0.0 | (4)1.0 ± 0.0 | (6)1.2 ± 0.0 | (6)2.4 ± 0.5 | ..... |
| 7 | Increased amount of distillate collected | (6)1.7 ± 0.1 | (8)0.75 ± 0.23 | (8)1.4 ± 1.1<br>(2) > 4.0 | (6)1.4 ± 0.7 | ..... |
| 8 | No pretreatment | (6)1.4 ± 0.3 | ..... | (4)1.5 ± 0.0 | (4)2.4 ± 0.1 | ..... |
| 9 | Petroleum ether used as extracting solvent | (8)1.6 ± 0.2 | ..... | (10)1.8 ± 0.3 | (2) > 4.0<br>(4)2.6 ± 0.4 | (2) > 585<br>(4)380 ± 60 |
| 10 | Anhydrous $Na_2SO_4$ used for drying ether extract | (8)1.6 ± 0.2 | ..... | (6)1.3 ± 0.3 | (6) > 4.0<br>(2)3.0 ± 0.0 | (6) > 15.7<br>(2)11.8 ± 0.0 |
| 11 | Ether-soluble volatile fraction vacuum desiccated | (6)1.2 ± 0.6 | ..... | (6)2.6 ± 1.1 | (8)2.0 ± 0.8 | (8) 6.2 ± 1.9 |

[a] Details described in full in text.

[b] The numbers in parentheses indicate the number of judgments. Rejected judgments are not included.

experiments are reported in table 1 and discussed individually in the following paragraphs.

In experiment no. 1, steam at atmospheric pressure (742 mm. of mercury) was passed through 60 g. of stale butter oil in the distilling flask and collected in 60 g. of butter oil prepared by melting and centrifuging freshly churned butter at 40° C. The receiver was maintained at approximately 40° C. by immersion in a constant-temperature water bath. After 200 ml. of distillate were collected in 1.75 hr., the contents of both the distilling flask and the receiver were separated and the water layers discarded. In the case of the residual butter oil in the distilling flask, it was necessary to centrifuge for 5 to 15 min. at 40° C. and 900 r.p.m. in order to break the emulsion formed during distillation. The original, residual and receiver butter oils were blended with fresh skim milk and fresh whole milk and their stale-flavor threshold values determined in the manner described previously (1). The stale flavor was detected in the receiver butter oil.

To determine whether this treatment in itself might be sufficiently vigorous to cause any flavor changes in the receiver butter oil, it was repeated in experiment no. 2 at an atmospheric pressure of 731–732 mm. of mercury with fresh butter oil replacing the stale butter oil in the distilling flask. No off-flavors were observed after distillation in either the residual or receiver butter oils when judged in the usual manner.

Since the judges comments on experiment no. 1 indicated some flavor changes in the residual butter oil which were not considered desirable, experiment no. 3 was performed under reduced pressure (68 to 74 mm. of mercury) so that the stale butter oil in the distilling flask would not be subjected to high temperatures. The temperature of the vapor before condensing did not rise above 51° C. Approximately 300 ml. of distillate were collected in 1.75 hr. Upon blending and scoring, the stale flavor again was detected in the receiver oil.

It was thought that the stale-flavor component detected in the fresh butter oil might be due to volatile catalysts from the stale butter oil which were accelerating the formation of stale-flavor components in the fresh butter oil during the distillation process. To eliminate this possibility, experiment no. 4 was performed with 60 ml. of distilled water maintained at 0° C. replacing the fresh butter oil in the receiver. After approximately 300 ml. of distillate had been collected in 2 hr. under reduced pressure (67–68 mm. of mercury) at a temperature of 34 to 38° C., fresh butter oil was added to the distillate in a separatory funnel at 40° C. and agitated for 2 min. The water layer was discarded and the butter oil blended and scored in the usual manner. The stale flavor was observed in this butter oil extract of the distillate.

Experiment no. 5 was performed in the same manner as experiment no. 4, except under atmospheric pressure (745 mm. of mercury). The stale flavor was again noted in the fresh butter oil which had been used to extract the distillate.

Since, in all previous experiments, consideration of the threshold values of the various stale butter oils indicated the possible synthesis of stale-flavor component in the residual butter oils during distillation, it was thought that this

phenomenon could be used to increase the concentration of stale-flavor component in the stale butter oil before distillation and thus improve the yield in the distillate. Preliminary tests indicated that a statistically significant decrease in the stale-flavor threshold value of the stale butter oil could be obtained by refluxing steam under reduced pressure through the butter oil for 6 hr. Therefore, in experiment no. 6, 120 g. of stale butter oil were placed in a round-bottom flask equipped with a vertical condenser and the flask was immersed in a water bath at 48 to 49° C. Then steam under reduced pressure was refluxed through this butter oil for 6 hr. The pressure was maintained at 62 to 67 mm. of mercury by a pump attached to the outlet of the reflux condenser, and the boiler temperature was held at 43 to 45° C. A 50-g. sample of the pretreated butter oil was removed, blended with fresh skim milk and fresh whole milk and scored in the usual manner. The balance of the butter oil was stored overnight at –26° C. On the following morning, it was steam distilled under reduced pressure (56–87 mm. of mercury) at 30 to 44° C. as in experiment no. 4. A statistically significant increase in the concentration of stale-flavor component was observed in the pretreated butter oil, but no improvement in the yield was obtained in the distillate.

These results seemed to indicate that the steam as it passed through the stale butter oil became saturated with the stale-flavor component under the condition of these experiments and that, in order to increase the yield, a longer distillation period would be required. Therefore, the procedure of the preceding experiment was repeated in experiment no. 7, except that the volume of distillate collected was doubled. In the pretreatment, the pressure was maintained at 66 to 70 mm. of mercury and the boiler temperature was 44.5 to 47° C. During distillation, the pressure was 66 to 72 mm. of mercury and the temperature of the vapor before condensing was 43.5 to 48° C. No statistically significant differences were observed between the threshold values of the corresponding fractions in this and the preceding experiment. A considerable variation in the threshold of the judges was noted and a number of other off-flavors were reported in these two experiments, indicating that the treatment probably was too vigorous for the purpose of this study.

In experiment no. 8, the method of experiment no. 7 was repeated without the pretreatment. The pressure was maintained at 50 to 85 mm. of mercury and the temperature before condensing was 40 to 47° C. No significant decrease in the stale-flavor threshold value for the fresh butter oil extract of the distillate could be observed when the larger volume of distillate collected was compared with the smaller volumes of previous experiments.

To determine the weight of the stale volatile fraction distilled from the stale butter oil, some modifications were necessary. In experiment no. 9, the steam distillation was performed at 54 to 62 mm. of mercury pressure with the temperature of the distillate before condensing at 40 to 42° C. The amount of distillate collected in 4.4 hr. in 60 g. of distilled water in the receiver was 287.6 g. After distillation, the contents of the condenser system were rinsed into the receiver with petroleum ether which previously had been redistilled. Then the

distillate was extracted by agitation with three successive 100-ml. portions of petroleum ether for 2 min. each. The petroleum ether extracts were combined and stored overnight at ~7° C. On the following morning, the solvent was removed at room temperature by a water aspirator. The residual colorless oil, which gradually changed to a white amorphous material, weighed 878.4 mg. It then was dissolved in 50 g. of fresh butter oil at 40° C. and was blended and scored in the usual manner. The stale flavor was detected in the mixture at approximately the same threshold value as that of the fresh butter oil extracts of the distillates in the previous experiments.

The unexpectedly large weight of the petroleum ether-soluble volatile fraction obtained in the previous experiment indicated the possibility that some water had been transferred with the petroleum ether. Therefore, the procedure was modified in experiment 10 so as to dehydrate the petroleum ether extract before removing the solvent. The distillation was performed under a pressure of 52 to 62 mm. of mercury with the vapor temperature before condensing at 39 to 42° C. In 3 hr., 303.1 g. of distillate were collected in 60 g. of distilled water and extracted with petroleum ether as before. Then 2 g. of anhydrous $Na_2SO_4$ were added to the extract before storing it overnight at ~7° C. On the following morning, the extract was filtered to remove the $Na_2SO_4$ before removing the solvent. The weight of the petroleum ether-soluble volatile fraction (23.6 mg.) was reduced to approximately 1/37th of that obtained in experiment no. 9, but a considerable loss of the stale-flavor component was indicated when the fraction was dissolved in fresh butter oil, blended and scored in the usual manner.

The addition of anhydrous $Na_2SO_4$ to the petroleum ether extract therefore was omitted in experiment no. 11. The distillation was performed under a pressure of 68 to 71 mm. of mercury with the vapor temperature before condensing at 38 to 48° C. In 1.2 hr., 397 g. of distillate was collected in 64.3 g. of distilled water. After extraction with petroleum ether as before, the solvent was removed immediately from the extract by aspiration and the residue was stored for 60 min. in a vacuum desiccator over filter-paper strips impregnated with paraffin wax. No apparent loss of stale flavor was noted when it was dissolved in fresh butter oil, blended and scored.

## DISCUSSION

When the results reported in table 1 are examined with respect to the volatility of the stale-flavor component with steam, the evidence is quite clear. No serious flavor changes were produced in fresh butter oil when subjected to this treatment in experiment 2. Thus the results of experiments 1 and 3 can be interpreted as indicating that either the stale-flavor component is volatile with steam at atmospheric or reduced pressures or that volatile catalysts are present significant increase was noted in he stale-flavor threshold value of the fresh butter oil sufficiently to produce a detectable concentration by the end of the distillation period. However, in experiments 4 and 5, when distilled water replaced the fresh butter oil in the receiver with the resulting reduction in the time of ex-

posure of the fresh butter oil to these possible volatile catalysts, no statistically significant increase was noted in the stale-flavor threshold value of the fresh butter oil containing the volatile fraction. Therefore, the stale flavor present in this fresh butter oil was not synthesized in it but rather was in the distillate from the stale butter oil in the distilling flask. Experiments 9 and 11 further this conclusion by isolating a weighable petroleum ether-soluble volatile fraction which, when dissolved in fresh butter oil and blended and scored in the usual manner, possessed a detectable stale flavor when this fraction was present in concentrations as low as 6.2 parts per million. In only one experiment (no. 10) were the majority of the judges unable to detect the stale flavor in this fraction at its highest concentration and this may possibly be attributed to the presence of the anhydrous $Na_2SO_4$, which may have reacted with or adsorbed the stale-flavor component. A comparison of the weights and the stale-flavor threshold values of the petroleum ether-soluble volatile fractions obtained in experiments 10 and 11, indicates that the stale-flavor component is by no means all of this fraction. The fraction secured in experiment 10, although actually the heavier, contained less stale flavor or perhaps none at all.

When these results are considered to establish the best steam distillation procedure, the evidence is not particularly clear, due to the difficulty in obtaining uniformity in the stale-flavor threshold values. However, some generally desirable conditions are indicated. In all experiments, the stale-flavor threshold values of the residual stale butter oil are lower than would be calculated from the threshold values of the original butter oils and fresh butter oils plus distillate. Also in many experiments, especially in 1 and 6, in which more vigorous treatments were used, comments made by the judges indicated the presence of other off-flavors. Therefore, distillation conditions should be as mild as possible. The time of distillation should be not more than 2 hr. The temperature of the stale butter oil should be as low as possible (40 to 50° C.). The exposure to atmospheric oxygen during distillation should be at a minimum. These conditions can be approached by performing the steam distillation in the apparatus described under approximately 70 mm. of mercury pressure with the collection of about 300 ml. of distillate. While refluxing the stale butter oil with steam under reduced pressure apparently increases the concentration of stale-flavor component in the butter oil, it is probably not desirable since no measurable increase in yield in the distillate was obtained when this was done in experiments 6 and 7 and other off-flavors may be produced by the extra treatment. Even though the stale-flavor component is not as soluble in water as in butter oil, a comparison of the stale-flavor threshold values of the fresh butter oil plus distillate fraction in the various experiments indicates that distilled water at 0° C. is as effective a receiving liquid as fresh butter oil at 40° C. The results of experiments 9 and 11 indicate that the stale-flavor component can be extracted from the distillate by petroleum ether without appreciable loss. This petroleum ether extract should constitute a very useful solution for further fractionation in the isolation of the stale-flavor component.

## SUMMARY

The possibility of separating the stale-flavor component from stale butter oil by steam distillation was investigated.

A procedure was developed by which a fraction was separated from the stale butter oil. This fraction, when dissolved in fresh butter oil and blended with fresh products to the composition of the original milk, yielded a detectable stale flavor in concentration as low as 6.2 parts per million.

This procedure involves the steam distillation of stale butter oil under a pressure of 70 mm. of mercury and at a temperature of approximately 45° C. The distillate is collected in water at 0° C. and the aqueous suspension is extracted with petroleum ether. The fraction containing the stale flavor then is obtained by evaporating the petroleum ether.



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# IMPORTANCE OF HAY QUALITY AS INDICATED BY FEEDING TRIALS WITH IDENTICAL TWIN DAIRY HEIFERS[1,2]

CHANDRAKANT N. KELKAR,[3] AND T. W. GULLICKSON
*Division of Dairy Husbandry, University of Minnesota, St. Paul*

Hay quality in relation to its feeding value for cattle has received much attention in recent years. Chemical analyses generally have shown that the higher grades of hay are lower in fiber but richer in protein, carotene and minerals than lower grades of the same kind of hay. Also, in feeding tests the higher grade hay generally has been found to be more palatable. However, only a few trials have been reported concerning the feeding value of different grades of hay for dairy heifers. Cary (2) reported that calves fed normal rations including U. S. no. 3 or poorer alfalfa hay must be given a vitamin A supplement if they are to survive. Morrison (5) states that poor quality hay supplemented with vitamin A may not be as good as similar rations containing high grade hay. Others (3, 7) have indicated that heifers cannot be raised satisfactorily on an exclusive ration of even high grade legume hay, but no harmful effects on reproduction or sexual development were reported.

## EXPERIMENTAL

The experiment was designed to compare the feeding value of U. S. no. 1 extra leafy green with U. S. no. 3 leafy brown alfalfa hay in feeding trials, utilizing two sets of identical twin heifers. In such animals the germ plasma is derived from a common zygote. Bonnier and Hanson (1) state that identical twins are at least 20 times as efficient as non-twins for use in group experiments involving growth. New Zealand workers (4) reported that one pair of identical twins is equivalent to 100 ordinary cows in studies involving milk production and 25 calves in growth studies.

Before any twins were placed on experiment the animals within each set were subjected to a rigorous comparison to establish their monozygosity. Comparisons were made for similarity in major details of color, markings, hair whorls, number of teats, shape and development of udders, nose prints, temperaments, feed intakes, weights, heights at withers, rate of gains in weight and height, blood types and hemoglobin values when fed identical rations.

The two sets of identical twin heifers selected were: T11 and T12, grade Holsteins, 287 days old, and T18 and T19, 393 days old, the offspring of a grade

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[3] Present address: Ramdas Peth, Nagpur, C. P., India.

Holstein dam and a purebred Red Polled sire. T11 and T12 resembled Holsteins in type and T18 and T19 closely resembled their sire in type but were black and white in color.

The experiment started on November 21, 1948, and continued for 280 days. One heifer from each pair, *i.e.*, T11 and T18, was fed U. S. no. 1 extra leafy green alfalfa hay as her sole ration and the twin sisters T12 and T19, respectively, were fed U. S. no. 3 leafy brown alfalfa hay, with T19 also being fed such amounts of a concentrate mixture as would enable her to gain at the same rate as T18. The concentrate mixture was made up of 225 lb. ground yellow corn, 300 lb. each of ground oats and ground barley, 75 lb. each of soybean oil meal and linseed meal, 15 lb. salt and 10 lb. steamed bone meal. According to the table by Morrison (5) showing the average composition and digestible nutrients in feeds, the mixture contained approximately 12.4 per cent digestible protein and 74 per cent total digestible nutrients.

The heifers when in the barn were kept in stanchions. They were fed at 7 a.m. and at 5 p.m. daily and each heifer always was fed slightly more hay than she would consume. They also had free access to clean water and a mineral mixture made up by weight of three parts steamed bone meal and one of salt. Records were kept of the amounts of feed fed to, and consumed by, each heifer daily. The heifers were turned outdoors for exercise during the day whenever weather permitted.

Composite samples were collected from both grades of alfalfa hay when fed. The results of the chemical analyses of the samples are shown in table 1. The

TABLE 1

*Chemical analysis and digestible nutrient content of both grades of alfalfa hay fed*

| Alfalfa hay | Dry matter | Crude | | Ether extract | N.-free extract | Carotene | Digestible protein | Total digestible nutrients |
|---|---|---|---|---|---|---|---|---|
| | | protein | fiber | | | | | |
| | (%) | (%) | (%) | (%) | (%) | (*mg./100 g.*) | (%) | (%) |
| U. S. no. 1 extra leafy green | 87.2 | 16.1 | 27.0 | 1.8 | 34.5 | 1.9 | 11.9 | 49.7 |
| U. S. no. 3 leafy brown | 90.4 | 14.1 | 35.1 | 1.4 | 32.7 | 0.4 | 9.4 | 46.7 |

digestion coefficients reported by Morrison (5) for alfalfa hay of corresponding crude fiber contents were used in calculating the digestible protein and the total digestible nutrient contents of the two grades of hay.

Each heifer was weighed every Sunday morning before she was fed and measurements for height at withers were made every 4 wk. Records were kept of dates when each heifer showed estrum and when bred. General observations in regard to health and appearance of animals were made daily.

## RESULTS

The weight and height at withers of each heifer at the start and at the end of the 280-day period, as well as her total gain in weight and height and average daily gain in weight, are shown in table 2. The rate of gain in weight compared

with the Ragsdale standard (6) for Holsteins also is indicated. The data show that all four heifers gained in weight at above the normal rate. Facts concerning the average weight of each heifer, average daily feed and nutrient intake, average daily nutrient requirement according to the Morrison standard (5) and

TABLE 2

*Data concerning the weight and height at withers of each heifer*

| Herd no. | Weight at | | Height at withers at | | Gain in weight | | | Total gain in height |
|---|---|---|---|---|---|---|---|---|
| | Start | End | Start | End | Total | Daily | Per cent of normal[a] | |
| | (*lb.*) | (*lb.*) | (*cm.*) | (*cm.*) | (*lb.*) | (*lb.*) | | (*cm.*) |
| T11 | 517 | 924 | 113.6 | 130.1 | 407 | 1.45 | 119.4 | 16.5 |
| T12 | 518 | 887 | 114.1 | 128.2 | 369 | 1.32 | 108.2 | 14.1 |
| T18 | 582 | 951 | 109.7 | 119.2 | 369 | 1.32 | 112.5 | 9.5 |
| T19 | 585 | 965 | 109.9 | 120.4 | 380 | 1.36 | 115.9 | 10.5 |

[a] Growth Standards for Dairy Cattle (6).

the percentage which the T.D.N. intake is of the amount required, during successive 56-day intervals and for the entire period of the experiment are presented in table 3.

The data in table 1 reveal that, pound for pound, the U. S. no. 1 extra leafy green alfalfa hay contained approximately 1.3 times as much digestible protein, 1.1 times as much T.D.N. and nearly 5 times as much carotene as the U. S. no. 3 leafy brown alfalfa hay. The U. S. no. 1 extra leafy green alfalfa hay also was considerably more palatable than the U. S. no. 3 leafy brown alfalfa hay, as indicated by the fact that the daily hay consumption by T11 was about 3.5 lb. greater than for T12.

The superiority of the no. 1 over the no. 3 grade of alfalfa also is suggested by the fact that T11 gained 38 lb. more in weight and 2.4 cm. more in height at withers than T12 during the period of the experiment. These differences, however, are small when the great difference between the nutrient intakes of the two animals is considered. T12 actually received less than 80 per cent as many pounds of T.D.N. daily (2.2 lb. less) than T11. Compared with the Morrison standard (5) T11 and T12 received 106 and 86 per cent, respectively, of their T.D.N. requirements for growth. T12 also received only about 75 per cent of the required amount of carotene. These results suggest that a more efficient utilization of nutrients received was made by T12 than by T11. Morrison (5) states, "there is even more difference in the utilization of the digested nutrients in the liberal and scanty rations than there is in the percentage digested."

Comparisons like the foregoing cannot be made between T18, fed U. S. no. 1 extra leafy green alfalfa hay, and T19, fed U. S. no. 3 leafy brown alfalfa hay, because the latter, as has been stated, was fed enough concentrates along with the hay to enable her to gain in weight at the same rate as T18. T19 consumed an average of 4 lb. concentrates daily and derived approximately 31 per cent of her T.D.N. intake from this source. The data in table 3 show that the daily

T.D.N. intake was approximately the same for both heifers and, as they made the same gains in weight, it is apparent that the nutrients supplied in both rations were equally well utilized. There was no significant difference between the two heifers in gain in height at withers.

All four heifers received an adequate supply of protein, but the rations of T12 and T19, fed the lower grade hay, were deficient in carotene; however,

TABLE 3

*Data concerning the average weight, daily feed and nutrient intakes and nutrient requirements of each heifer during successive 8-wk. periods and for the entire period of the experiment*

| 8-wk. period no. | Av. weight | Feed consumed | | Nutrient intake | | | Nutrients required[b] | | | Required T. D. N. provided |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Alfalfa hay | Concentrate | Protein | T.D.N. | Carotene[a] | Protein | T.D.N. | Carotene | |
| | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) | (*mg.*) | (*lb.*) | (*lb.*) | (*mg.*) | (%) |
| | | | | | T 11 | | | | | |
| 1 | 562 | 17.5 | ..... | 2.1 | 8.7 | 150 | 0.88 | 8.1 | 34 | 107.4 |
| 2 | 666 | 19.3 | ..... | 2.3 | 9.6 | 166 | 0.92 | 9.0 | 40 | 106.7 |
| 3 | 765 | 21.5 | ..... | 2.6 | 10.7 | 185 | 0.94 | 9.7 | 46 | 110.3 |
| 4 | 845 | 22.4 | ..... | 2.7 | 11.1 | 193 | 0.96 | 10.2 | 51 | 108.8 |
| 5 | 896 | 22.2 | ..... | 2.6 | 11.0 | 191 | 0.98 | 10.5 | 50 | 104.8 |
| Av. | 747 | 20.6 | ..... | 2.5 | 10.2 | 178 | 0.94 | 9.6 | 45 | 106.3 |
| | | | | | T 12 | | | | | |
| 1 | 552 | 13.0 | ..... | 1.2 | 6.1 | 24 | 0.88 | 8.0 | 33 | 76.3 |
| 2 | 634 | 16.7 | ..... | 1.6 | 7.8 | 30 | 0.91 | 8.8 | 38 | 88.6 |
| 3 | 722 | 18.2 | ..... | 1.7 | 8.5 | 33 | 0.93 | 9.4 | 43 | 90.4 |
| 4 | 798 | 19.6 | ..... | 1.8 | 9.2 | 36 | 0.95 | 9.9 | 48 | 92.9 |
| 5 | 855 | 18.7 | ..... | 1.8 | 8.7 | 34 | 0.97 | 10.2 | 48 | 85.3 |
| Av. | 712 | 17.2 | ..... | 1.6 | 8.0 | 31 | 0.93 | 9.3 | 43 | 86.0 |
| | | | | | T 18 | | | | | |
| 1 | 623 | 17.3 | ..... | 2.1 | 8.6 | 149 | 0.90 | 8.7 | 37 | 98.9 |
| 2 | 688 | 17.6 | ..... | 2.1 | 8.7 | 152 | 0.92 | 9.2 | 41 | 94.6 |
| 3 | 779 | 19.6 | ..... | 2.3 | 9.7 | 169 | 0.95 | 9.8 | 47 | 99.0 |
| 4 | 855 | 20.4 | ..... | 2.4 | 10.1 | 176 | 0.97 | 10.2 | 51 | 99.0 |
| 5 | 918 | 21.6 | ..... | 2.6 | 10.7 | 186 | 0.98 | 10.6 | 55 | 100.9 |
| Av. | 773 | 19.3 | ..... | 2.3 | 9.6 | 166 | 0.94 | 9.7 | 46 | 99.0 |
| | | | | | T 19 | | | | | |
| 1 | 618 | 14.9 | 1.29 | 1.6 | 7.9 | 27 | 0.90 | 8.6 | 37 | 91.9 |
| 2 | 685 | 15.0 | 3.71 | 1.9 | 9.8 | 27 | 0.92 | 9.1 | 41 | 107.7 |
| 3 | 770 | 15.3 | 4.50 | 2.0 | 10.5 | 28 | 0.94 | 9.7 | 46 | 108.2 |
| 4 | 855 | 13.5 | 5.46 | 1.9 | 10.3 | 25 | 0.97 | 10.2 | 51 | 101.0 |
| 5 | 925 | 10.6 | 5.00 | 1.6 | 8.7 | 19 | 0.98 | 10.6 | 55 | 82.1 |
| Av. | 771 | 13.9 | 3.99 | 1.8 | 9.4 | 25 | 0.94 | 9.7 | 46 | 96.9 |

[a] Carotene in concentrates not included.
[b] Morrison (5).

neither of these heifers exhibited symptoms of a vitamin A deficiency at any time during the period of the experiment. The physical appearance of the heifers fed no. 1 alfalfa hay was in general slightly superior throughout to that of those fed no. 3 alfalfa hay.

It is significant that there was as great a difference between the gains in weight of T11 and T18, both of which were fed no. 1 alfalfa hay, as between T11

and her twin sister T12, fed no. 3 alfalfa hay. These results illustrate how the individuality of animals selected may affect experimental results. By using identical twins this upsetting factor largely is eliminated.

The evidence is incomplete in regard to the possible effect of quality of hay fed on sexual development of heifers. The records show that T18 and T19 were sexually mature when started on experiment. T18 came in heat and was bred on December 1, 1948, and T19 was bred on December 20. Both heifers were bred to the same sire and each conceived from a single service. T11 first came in heat December 29, 1948, but T12 did not show estrum until 96 days later. After the first heat period, however, each heifer showed estrum regularly. T12 was first bred on May 17, 1949, and T11, 11 days later. Since that time both heifers were bred regularly at each heat period but neither of them conceived. No explanation other than difference in quality of hay fed is available to account for the delay in appearance of estrum in T12.

## CONCLUSIONS

The U. S. no. 1 extra leafy green alfalfa hay was more palatable, richer in digestible nutrients and carotene and, when fed to heifers, produced slightly more rapid gains in weight and in height at withers than U. S. no. 3 leafy brown alfalfa hay, but at a higher cost in nutrients per unit of gain.

The value of using identical twins in experiments comparing the nutritive value of feeds is indicated.

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# THE NUMBER OF PROVED SONS NECESSARY TO EVALUATE THE TRANSMITTING ABILITY OF A DAIRY SIRE [1, 2]

W. E. WASHBON[3] AND W. J. TYLER
*West Virginia University, Morgantown*

Breeders of dairy cattle constantly are looking for more aids to use in selecting future herd sires. Washbon (2, 3) has advocated the use of the performance of a sire's proved sons (based on Dairy Herd Improvement Association records) as a basis for evaluating the transmitting ability of the sire and has suggested selection of bulls whose paternal brothers have sired daughters that produce more butterfat than their dams at a relatively high level of production.

The reliability of selecting bulls by this method depends upon how well the later proved sons of a sire repeat the performance of their earlier proved paternal brothers. This paper reports the results of a study of the correlation between the daughter performance of varying numbers of first proved and later proved paternal brothers.

## EXPERIMENTAL PROCEDURE

One hundred seventy-four Holstein sires with eight or more DHIA proved sons were studied to determine the least number of proved sons necessary to estimate most accurately the performance of those to be proved later. The data included: (a) average butterfat production of the proved sons' daughters, (b) average differences of daughters' production as compared to their dams and (c) the per cent of proved sons that maintained or increased butterfat production of the herds in which they were used. The average performance of the first three to ten proved sons, respectively, was compared with the performance of the following five and ten sons.

The essential information about each proved son of the 174 Holstein sires studied was punched on International Business Machine cards. These cards, approximately 3,000 in number, then were sorted by sires either in actual sequence of proof, if known, or in sequence of registration. The cards were arranged so as to obtain the listing and totals of the first three sons, the next five sons and the next ten sons for each of the 272 sires. The cards then were rearranged by placing the first four sons in the first group, to be compared with the following five and ten sons. This was repeated for the first five, six, seven, eight, nine and ten sons where sufficient numbers made comparisons possible.

The essential data and the averages of each group and of the groups with

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[3] Now Cattaraugus County Agricultural Agent, Salamanca, New York.

which each was to be compared were computed and punched upon summary cards. The summary cards were used to obtain listings of comparative performance of associated groups of sons. The summary cards again were used to obtain sums of squares and cross products for statistical analysis of the data.

A difference in the average number of dam-daughter comparisons between the first proved sons and those proved later might affect the conclusions drawn from a comparison of two groups of paternal brothers. A study involving 118 Holstein sires with 1,782 proved sons indicated that there was no appreciable difference in the average number of dam-daughter comparisons between groups of the data to be studied.

A consistent but slight tendency for the better sons of a sire to be proved more promptly than the poorer ones was indicated by a consistently higher fat level and daughter fat difference of first proved sons arranged by actual order of proof as compared to the same sons arranged in sequence of registration. The extent of this influence was not considered sufficient to affect materially the investigation.

## RESULTS

The average butterfat produced by the daughters of the first three to ten proved sons was 386 lb., which was 10 lb. less than the average production of the daughters of the next five or ten proved sons (Table 1). This greater production of the later proved sons' daughters is ascribed largely to the gradual improvement of economic conditions from 1930 to 1948 which favored daughters of the later proved sons. The standard deviation of the average daughter fat of the groups of proved sons ranged from 40 lb. of fat with three sons in the group to 25 lb. when the group contained from six to ten sons.

TABLE 1

*Means and regression coefficients (b) of mean daughter fat of later proved sons upon the means of three, five and ten sons proved earlier*

| First proved sons | | Next 5 sons | | Next 10 sons | |
|---|---|---|---|---|---|
| Number | Mean | Mean | *b* | Mean | *b* |
| 3 | 385 | 391 | 0.66 | 395 | 0.55 |
| 5 | 387 | 394 | 0.62 | 393 | 0.59 |
| 10 | 384 | 400 | 0.60 | 395 | 0.37 |

The correlation coefficients between average daughter butterfat of the first proved Holstein sons and those proved later were statistically significant and ranged from 0.40 to 0.65, as shown in table 2. Correlations decreased as the average daughter butterfat of the first eight, nine and ten proved sons was compared to the performance of the daughters of the next ten. This was the opposite of normal expectation, since a comparison involving larger numbers would be expected to show higher correlations than smaller numbers. This trend did not exist when comparing the first three to ten proved sons, respectively, with the next five sons. Weighted average correlations (1) of sons' daughters' average butterfat between the first three to ten proved sons, inclusive, and the next

TABLE 2

*Correlation coefficients between average performance of first proved sons and those proved later of Holstein sires*

| First sons proved | Correlation coefficients between butterfat level of the daughters of first and later proved sons | | | | Correlation coefficients between fat difference of the daughters of first and later proved sons | | | | Correlation coefficients between per cent of sons plus of first and later proved sons | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of comparisons | Next 5 sons | No. of comparisons | Next 10 sons | No. of comparisons | Next 5 sons | No. of comparisons | Next 10 sons | No. of comparisons | Next 5 sons | No. of comparisons | Next 10 sons |
| 3 | 174 | 0.50** | 72 | 0.62** | 174 | 0.30** | 72 | 0.31** | 174 | 0.15* | 72 | 0.12 |
| 4 | 148 | 0.46** | 59 | 0.51** | 148 | 0.19* | 59 | 0.28* | 148 | 0.08 | 59 | 0.31* |
| 5 | 129 | 0.45** | 52 | 0.56** | 129 | 0.10 | 52 | 0.35** | 129 | 0.00 | 52 | 0.32* |
| 6 | 103 | 0.53** | 46 | 0.65** | 103 | 0.35** | 46 | 0.40** | 103 | 0.17 | 46 | 0.31* |
| 7 | 87 | 0.50** | 41 | 0.54** | 87 | 0.29** | 41 | 0.32* | 87 | 0.04 | 41 | 0.25 |
| 8 | 72 | 0.48** | 34 | 0.49** | 72 | 0.36** | 34 | 0.24 | 72 | 0.12 | 34 | 0.13 |
| 9 | 59 | 0.48** | 29 | 0.45* | 59 | 0.30* | 29 | 0.04 | 59 | 0.25 | 29 | 0.01 |
| 10 | 52 | 0.64** | 27 | 0.40* | 52 | 0.26 | 27 | 0.04 | 52 | 0.24 | 27 | 0.04 |
| Weighted av. $r$ | | 0.50** | | 0.55** | | 0.26** | | 0.28** | | 0.11 | | 0.21 |

* $0.01 < P < 0.05$ = Significant.
** $P < 0.01$ = Highly significant.

five and next ten sons were computed and are given in the bottom line of table 1.

The regression coefficients of mean daughter fat of later proved sons upon those proved first ranged from 0.32 to 0.85, most of them being in the range 0.45 to 0.60.

The correlation coefficients between the mean daughter fat difference of the first proved sons and those proved later ranged from 0.04 to 0.40 and are shown in table 1. The weighted average correlation between the first three to ten proved sons, inclusive, and the next five sons was 0.26, while a weighted correlation of 0.28 was obtained in the comparison between the first three to ten proved sons and the performance of the next ten sons.

The average per cent of sons maintaining or increasing production does not change materially as more sons are proved. There was some indication of a trend toward a lower percentage as more than 15 sons were proved.

The correlation coefficients between the average per cent sons plus of the first proved sons and those proved later are given in table 1. The average weighted correlation between the first three to ten sons, inclusive, and the next five sons was 0.11, while a correlation of 0.21 was obtained between the first group and the next ten sons.

## DISCUSSION

The variations between groups of sons of the same sire are a result of sampling of the sire's inheritance, differences in transmitting abilities of the dams of the sons, differences in the average inheritance of the cows to which the sons are mated, and differences in temporary or permanent environments affecting the herds in which the sons were used. A group of ten sons of a sire might be mated to dams that averaged as much as 75 lb. of fat above or below the dams to which another ten sons were mated. One group of sons could, by chance, be mated to dams of better inheritance than another group. These extreme conditions were rather uncommon within the data studied but did exist in a few instances.

There was some indication that the differences in the inheritance of the dams of the sons contributed to this variation. Among the 154 sires with sons arranged by order of registration number, there were several instances in which a higher proportion of first born sons increased or decreased production. This was followed by the opposite performance in later proved sons. It might happen that this relationship would occur purely by chance, although this is not to be expected. Partial explanation might lie in the often-followed policy of mating a young bull only to the daughters of an older sire at the farm. If the older sire possessed outstanding inheritance, the sons from such matings might be superior. As the young bull grew older, matings with the older females in the herd might give his later sons poorer inheritance than his first born sons because these new mates could be cows more representative of the herd average. The exact opposite of the situation is as likely in which the older sire was an outstandingly poor one.

The correlation coefficients indicated that the progeny performance of four or five proved sons was fairly reliable as a measure of a sire's transmission to future sons. When the first eight, nine and ten sons were compared with the next ten sons, the correlations were smaller than the correlations between the first three to seven sons and the next ten sons. However, the sampling error is large because of the small number of sires that had 18 or more sons, and may explain the differences observed here.

The method of averaging correlation coefficients was adopted because of the consistent nature of relationships from group to group. The weighted average correlation should give a slightly more reliable statistic than any individual correlation. The degrees of freedom for the weighted statistic will not be much larger than the largest number of degrees of freedom for any of the correlations that were averaged.

The correlation between one group of sons and the next group of sons by the same sire is a measure of the genetic similarities between the two groups plus the fact that the bulls in the two groups may have been used in herds with similar environment. Sires with proved sons performing at relatively high levels of production had groups of sons that fluctuated from that high average as much as groups of sons from mediocre sires varied from a mediocre average. When these son-group fluctuations were charted it was clear that the groups of sons from the superior sires fluctuated at a high level, the groups of sons from the mediocre sires varied around the breed average and the groups of sons from the poor sires fluctuated at levels below breed average. The pronounced tendency for groups of sons from superior sires to vary at consistently higher-than-average levels might allow more certain identification of desired inheritance provided the effects of similarities in herd environments for the sons of such sires can be evaluated. If stratification of herd environments is inherent in these data, then consideration of this factor should be taken into consideration if breeders are to select bulls on the basis of the performance of the bull's paternal brothers. This problem of evaluating the relative importance of herd environment and heredity needs further study.

## SUMMARY

A total of 174 Holstein-Friesian sires with eight or more D.H.I.A.-proved sons was studied to determine the least number of proved sons necessary to estimate accurately the performance of those to be proved later. The results showed that the average butterfat production of the sons' daughters increased as more sons were proven, a tendency that also was reflected in greater daughters' fat difference as compared to their dams. This increase probably was due more to environmental than genetic causes.

Highly significant correlations ($r = 0.46$ to $0.62$) indicated that the average butterfat production of the daughters of the first three or four sons was nearly as accurate as data on more sons in estimating the average butterfat production of the daughters of the next five or ten proved sons.

Similarly, the significant correlations ($r = 0.19$ to $0.31$) for the sons' daughters' average increase or decrease in butterfat production from their dams

indicated that data on the first three or four proved sons were nearly as accurate as data on a larger number in predicting what might be expected from the next five or ten proved sons in this respect.

The correlations for per cent sons improving production were lower than those for fat level or fat difference. Figures on the first three or four sons were as accurate as data on a larger number of sons in predicting percentage of improvers in the next five or ten proved sons.

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# FLAVOR DETERIORATION ASSOCIATED WITH THE LIPID PHASE OF WHOLE MILK POWDER[1]

ARTHUR T. MUSSET,[2] STUART PATTON AND CHESTER D. DAHLE
*Department of Dairy Husbandry, Pennsylvania Agricultural Experiment Station, State College*

Whole milk powder readily undergoes flavor deterioration, either during manufacture or shortly thereafter. This flavor defect is intensified with increased length of storage or elevated storage temperature. Definition of the defect includes such terms as "typical of whole milk powder," "heated," "like coconut," "stale" and various others. This defect should not be confused with oxidized flavor of dairy products, since it is detectable in whole milk powders of best quality long before true oxidized flavor normally is evident. Milk powder in which the milk fat has been replaced with a suitable vegetable fat appears to be free of this flavor (8).

Bailey (3) states, "Flavor reversion in fats is probably defined most satisfactorily as the appearance of objectionable flavor from less oxidation than is necessary to produce true oxidative rancidity." He points out that the amount of oxygen necessary to produce flavor deterioration in partially hydrogenated lard was only one-fiftieth of that necessary to produce (oxidative) rancidity. Bailey further believes "the respective phenomena of flavor reversion and rancidification are associated with amounts of oxidation so different in degree that it appears hardly proper to consider them more than casually related."

Some preliminary investigations (11) of flavor change in dry butterfat during storage indicated great similarity between the flavor developing in this medium and that produced in dry whole milk during its manufacture or shortly thereafter. West (15) observed this phenomenon when aged dry butterfat was re-emulsified in fresh, pasteurized skim milk. In addition, he found that the use of antioxidants and nitrogen packing, either together or separately, only delayed rather than prevented the onset of the "typical whole milk powder" flavor defect in dry butterfat stored as such. The development of this flavor in stored dry butterfat is catalyzed by heat and appears to resemble the mechanism of flavor deterioration in many other fats and oils.

Ashworth (1) reported that heating of the skim milk and cream separately prior to drying showed some promise of improving the shelf life of the resulting powder. Less flavor change was noted in powder made from concentrated skim milk receiving high heat treatment and cream receiving low heat treatment. A patent issued earlier to North and Alton (12) claims that the keeping quality of whole milk powders are enhanced if the cream is not subjected to condensing.

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[2] Present address: Beatrice Foods Co., Chicago, Ill.

Whitney and Tracy (16) recently have isolated a stale flavor component from dry whole milk which appears to be concentrated in the milk fat phase. These authors indicate that stale flavor develops only after some period of storage, whereas the flavor of interest in this report is that exhibited immediately after manufacture or a very short storage period.

This investigation was conducted to determine the effect of different fats on the initial and storage flavor qualities of dry whole milk. Secondly, a study of the storage characteristics of certain fractions of butter fat was made to determine which fractions are most concerned in the flavor deterioration of whole milk powders.

## EXPERIMENTAL

*Dry whole milk.* In order to study the contribution of the lipid phase of dry whole milk to flavor deterioration occurring at the time of manufacture or shortly thereafter, samples of dry whole milk were prepared utilizing the following fats: (a) Control (whole milk with fat untreated); (b) partially hydrogenated coconut fat (melting point 76° F.); (c) dry butterfat; and (d) a combination of 75 per cent partially hydrogenated cocoanut fat and 25 per cent dry butterfat. A quantity of high quality mixed herd milk was obtained from the college creamery and treated as follows: A portion of the milk was removed, standardized to 3.4 per cent fat, forewarmed to 170° F. for 30 min. and concentrated to approximately 36 per cent total solids. Upon completion of evaporation, the condensed milk was homogenized at 2500 p.s.i. through a single stage homogenizer, cooled to approximately 40° F. and held for drying the following day.

The remainder of the milk was centrifugally separated. The skim milk portion was forewarmed to 170° F. for 30 min. and condensed to approximately 29 per cent total solids, cooled and divided into three equal lots for addition and emulsification of fats. To one lot of the condensed skim enough partially hydrogenated coconut fat was added to give a fat/snf ratio equal to that of the control whole milk. Dry butterfat for addition to the second lot of condensed skim milk was prepared from the cream by a conventional method. Briefly, this consisted of pasteurizing (155° F. for 30 min.), cooling and churning the cream. The resulting butter was thoroughly washed, melted and rewashed several times with warm water. After the final washing, the wash water was removed by passing the oil-water mixture through an International Harvester separator. A mixture of 75 per cent partially hydrogenated coconut fat and 25 per cent butterfat was added to the third lot of condensed skim milk. The various fats were emulsified in the condensed skim milk by homogenization through a single stage at 2500 p.s.i.

The experimental batches of milk were dried in a Rogers chest-type spray drier which had four no. 72 atomizing nozzles and a capacity of 140 lb. of powder per hour. The milk was atomized at a pressure of 2500 p.s.i. and the outlet temperature of the drying chamber was approximately 186° F.

The powders were sifted and weighed into no. 1 tin containers. The tins

then were placed in a large container and subjected to a vacuum of 28 in. for 18 hr. At the end of this period, the cans were removed and sealed individually by means of an American Can Co. machine designed for automatic sealing under vacuum. The samples of powder were stored at room temperature. The fat ($27.85 \pm 0.40$ per cent) and moisture ($2.33 \pm 0.25$ per cent) content of all samples were relatively uniform.

Since there appeared to be no suitable chemical tests for measuring the flavor changes under consideration, and in view of the fact that several groups of investigators (4, 5, 6, 7, 9 and 10) have found organoleptic procedures the most useful for detecting flavor deterioration, such procedures were employed for evaluating flavor in these experiments. Five experienced judges were used at each taste session. Only one judge at a time was permitted to examine the samples. The identities of the samples were unknown to the judges. Criticisms of the samples were classified as slight, definite, pronounced and very pronounced. Flavor evaluations of the samples were made following manufacture and at monthly intervals thereafter.

*Dry butterfat fractions.* In an attempt to clarify the nature of flavor deterioration in dry butterfat, storage studies were conducted on various high and low melting butterfat fractions. The Winterization method of obtaining the desired fractions was selected over solvent crystallization as being least rigorous in treatment of the fat from a flavor viewpoint. This method has had commercial application in the vegetable oil and beef tallow industries for many years as a means of fractionating fats.

Dry butterfat was prepared from fresh sweet cream obtained from high quality milk of mixed herds on winter rations. The method of making the dry butterfat previously is described in this report. Prior to fractionation the butterfat was dried under vacuum to a moisture content of less than 0.1 per cent. After drying, the butterfat was divided into four equal lots which were treated as follows: Lot one was used as the control dry butterfat. The remaining three lots were adjusted to 32, 50 and 70° F., respectively, and allowed to crystallize for 24 hr. Upon completion of the crystallization period, each lot of liquid-solid mass was placed in a Carver hydraulic laboratory press for separation. The pressure on the mass was increased gradually by means of an hydraulic ram to 8000 p.s.i. and held at that pressure for several minutes. The liquid was collected and solids removed from the press for weighing and calculation of the per cent yield. Samples of the control butterfat along with each of the various liquid and solid fractions were air-packed in brown glass bottles for storage at room temperature. The melting points were determined on the fresh samples by the capillary tube method (2). Iodine numbers were ascertained by the Rosenmund-Kuhnhenn method (14).

By use of a hand homogenizer and fresh pasteurized skim milk, the samples of fat were re-emulsified on the basis of 4 per cent butterfat. Procedures for organoleptic evaluation of the re-emulsified samples were the same as those previously given for dry whole milk.

## DISCUSSION AND RESULTS

From a beverage standpoint, dry whole milk has certain inherent flavor defects. Although such defects may vary with manufacturing and storage conditions of the powder, they are evident in freshly-made dry milk of highest quality. The results of this study indicate that these initial flavor defects of dry whole milk have their origin, in part at least, in the lipid phase of the powder. The data in table 1 show clearly that a portion of the inherent flavor defects of whole

TABLE 1

*The effect of storage on the flavor of dry whole milks[a] made with various fats*

| Type of fat | Flavor after storage in months | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Control | + milk powder | ++ milk powder | ++ milk powder | ++ milk powder | +++ milk powder | +++ milk powder | +++ milk powder |
| Coconut fat | bland | bland | no criticism | no criticism | flat | flat[b] | flat |
| Dry butterfat | ++ milk powder | ++ milk powder | +++ milk powder | ++++ milk powder | ++ oxidized | ++ oxidized | +++ oxidized |
| 75% coconut fat 25% dry butterfat | bland | no criticism | no criticism | no criticism | flat | flat | stale |

[a] Double vacuum packed in no. 1 tins and stored at room temperature.
[b] Resembling raw potato flavor.
Code: + slight; ++definite; +++pronounced; ++++ very pronounced.

milk powder do not develop when part or all of the butterfat is replaced with hydrogenated coconut fat. These findings were confirmed by flavor data obtained independently at the Quartermaster Food Acceptance Laboratories on the same samples (13). It was observed further that the odor in the drying chamber following the processing of samples containing pure butterfat was typical of that associated with whole milk powder, whereas the milk containing hydrogenated coconut fat imparted little or no odor to the chamber.

The experiments dealing with flavor deterioration in dry butterfat, stored as such, also tend to support the observation that the lipid phase of dry whole milk is responsible for a portion of the characteristic flavor defect in the powder. The aged butterfat, when reconstituted in fresh skim milk, imparted a flavor very similar to that of dry whole milk (table 2). Certain of the taste panel members had difficulty in distinguishing between aged dry butterfat and dry whole milk when these products were reconstituted as beverage milk. The dry butterfat experiments also show that fat fractions obtained by the hydraulic expression of crystallized butterfat vary in their stability to storage deterioration. The solid fractions appear to be relatively stable in this respect, whereas the liquid fractions deteriorate rapidly. That the liquid fractions undergo considerable change during storage is borne out by significant variations in their iodine numbers (table 3), as well as by their flavor on reconstitution in fresh skim milk. The solid fat fractions seem to afford some protection of an antioxidant type to

TABLE 2

*The effect of storage[a] on the flavor of fat fractions obtained by the hydraulic expression of dry butterfat at various temperatures*

| Fractions | Age of samples | | | | | |
|---|---|---|---|---|---|---|
| | Fresh | 2 wk. | 1 mo. | 2 mo. | 3 mo. | 4 mo. |
| Control | no criticism | no criticism | + milk powder | ++ milk powder | +++ milk powder | +++ milk powder |
| 32° F. Liquid | ++ oxidized | +++ oxidized | ++++ oxidized | ..... | ..... | ..... |
| 32° F. Solid | no criticism | no criticism | no criticism | + milk powder | ++ milk powder | ++ milk powder |
| 50° F. Liquid | + milk powder | ++ oxidized | ++++ oxidized | ..... | ..... | ..... |
| 50° F. Solid | no criticism | no criticism | no criticism | no criticism | + milk powder | + milk powder |
| 70° F. Liquid | + milk powder | ++ oxidized | +++ oxidized | +++ oxidized | ++++ oxidized | ..... |
| 70° F. Solid | no criticism | no criticism | no criticism | + butterscotch | + butterscotch | + milk powder |

[a] Air packed in brown glass bottles at room temperature.
Code: + slight; ++ definite; +++ pronounced; ++++ very pronounced.

the liquid fractions, as evidenced by the relative stability of the control dry butterfat and a secondary control made by combining liquid and solid fat fractions immediately following expression.

TABLE 3

*Some properties of fat fractions obtained by the hydraulic expression[a] of crystallized dry butterfat at various temperatures*

| | Control | 32° F. | | 50° F. | | 70° F. | |
|---|---|---|---|---|---|---|---|
| | | Liquid | Solid | Liquid | Solid | Liquid | Solid |
| Melting Point (° *F.*) | 86–89 | 46–53 | 91–95 | 50–53 | 98 | 53–56 | 109 |
| Yield (%) | | 7 | 93 | 14 | 86 | 63 | 37 |
| Iodine Numbers | | | | | | | |
| Fresh | 33.0 | 36.2 | 31.9 | 39.9 | 29.9 | 36.3 | 25.2 |
| 4 mo. | 31.5 | 34.5 | 30.9 | 37.7 | 29.3 | 34.6 | 24.7 |
| Net change | −1.5 | −1.7 | −0.8 | −2.2 | −0.6 | −1.7 | −0.5 |

[a] 8000 p.s.i. pressure used.

## SUMMARY AND CONCLUSIONS

Studies of inherent and initial flavor changes produced during manufacture and early storage of dry whole milk indicate that deterioration of the lipid phase accounts, in part at least, for such changes. Milk powder prepared with hydrogenated vegetable fat was lacking in certain of the flavors normally present in dry milks containing butterfat. Further, aged dry butterfat upon reemulsification in fresh skim milk was observed to impart a flavor very similar to that typical of fresh dry whole milk of good quality. These findings were considered good evidence that dry butterfat may undergo certain initial changes in flavor prior to the onset of oxidative rancidity. Such changes in dry butterfat appear

to be catalyzed by heat and the presence of oxygen; however, they may take place at relatively low temperatures and oxygen levels during longer periods of time.

Fat fractions, obtained by hydraulic expression of butterfat crystallized at various temperatures, showed consistent variations in keeping quality. The solid fat fractions were all relatively stable with respect to flavor deterioration during storage, whereas all the liquid fractions deteriorated rapidly. It is suggested that preliminary deterioration of the low melting portion of butterfat may be responsible for the flavor changes observed in connection with dry whole milk and stored dry butterfat in this investigation.

The viewpoints and conclusions of this paper are not to be construed as approval of the authors for the use of substitute fats in dairy products. Substitute fats were used in these experiments as an academic approach to the question of flavor deterioration of a lipid nature in dry whole milk.

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# THE USE OF DEHYDRATED FORAGES IN DAIRY CATTLE RATIONS. I. GRAIN SUBSTITUTION WITH FINELY GROUND MATERIAL[1]

E. B. HOPE, R. E. ERB, T. H. BLOSSER, U. S. ASHWORTH AND A. O. SHAW[2]
*Division of Dairy Husbandry, Washington Agricultural Experiment Stations, State College of Washington, Pullman*

Numerous feeding trials have been conducted with milking cows and growing heifers using many different kinds of dehydrated forages. In these trials dehydrated forages clearly have shown a superiority for both growth and milk production over sun-cured forages harvested from the same field. Most of the earlier work on the nutritive value of dehydrated forages has been very well reviewed by Huffman (4) in 1939 and by Watson (13) in 1948.

Several workers have compared the value of dehydrated forages as a partial-to-complete replacement of concentrate mixtures for milk production. Woodman *et al.* (15), using dried grass protein cake, replaced all of the concentrate mixture and observed satisfactory milk and fat production. Watson (12) replaced the concentrate ration of five cows for 14 days with dehydrated grass without too much decline in production. Newlander (9) and Knott and Hodgson (5) replaced a portion of the concentrate mixture with dehydrated grass in change-over type experiments with short feeding periods without observing appreciable declines in milk production. Watson and Ferguson (14) fed an average of 8 lb. of dehydrated grass in meal form as a partial replacement for concentrates, using 5-wk. experimental periods. There was no statistical difference between milk, fat and solids-not-fat production when compared with controls. These workers further observed that the cows would eat no more than 8 to 10 lb. daily of this powdered material. In this connection, Cole and Mead (3), feeding finely ground alfalfa as the sole roughage, observed diminished rumination, depraved appetite and recurrent bloat. Camburn (2) replaced a part to all of a 20 per cent protein grain mixture with dried young grass, in a reversal type experiment with 3-wk. experimental periods, without observing any decline in milk production.

The purpose of the experiment herein reported was to test the grain replacevent value of a commercially prepared lot of dehydrated grass with high-producing dairy cows at 15, 30 and 45 per cent levels of grain replacement in a change-over type of experiment.

## EXPERIMENTAL

*Animals.* Twenty high-producing cows were divided into four groups consisting of three Holstein, one Jersey and one Guernsey per group. The age,

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stage of lactation, breeding date and production of each cow is shown in table 1. Cow 269 from group 2 and cow 300 from group 4 were removed from the experiment at the end of the first period and were replaced by cow 232 and cow 212, respectively. Cow 269 was suffering from rumen atony and possibly other undetermined ailments, and cow 300 died from a foreign body of long standing. Neither incident can be attributed to this experiment.

TABLE 1

*Age, date of freshening, productive level, date of breeding and group assignment of each cow used*

| Group | Herd no. | Breed | Age | Freshening date | 4% F.C.M. for first transition week | Date bred |
|---|---|---|---|---|---|---|
| | | | (*yr.*) | | (*lb.*) | |
| | 2124 | J | 2.5 | 3–26–48 | 223.0 | 11– 6–48 |
| | 3058 | G | 3 | 4–13–48 | 261.6 | 11– 8–48 |
| 1 | 237 | H | 7 | 4–29–48 | 449.7 | ..... |
| | 256 | H | 5.5 | 5–25–48 | 453.2 | 8–19–48 |
| | 274 | H | 4 | 10– 3–47 | 306.1 | ..... |
| Av. | | | | | 338.7 | |
| | 2125 | J | 2.5 | 3–27–48 | 188.7 | 12–24–48 |
| | 3900 | G | 4 | 6– 6–48 | 356.1 | 11–24–48 |
| | 207 | H | 9 | 10– 1–47 | 315.3 | ..... |
| 2 | 296 | H | 2.5 | 4–16–48 | 295.7 | 11– 7–48 |
| | 269[a] | H | 4.5 | 3–28–48 | 336.5 | ..... |
| | 232[b] | H | 7.5 | 7– 1–48 | 371.9 | ..... |
| Av.[c] | | | | | 305.5 | |
| | 2122 | J | 3 | 3– 4–48 | 201.4 | 11– 2–48 |
| | 3903 | G | 4 | 4–12–48 | 269.2 | ..... |
| 3 | 297 | H | 2.5 | 4–24–48 | 296.0 | 7–13–48 |
| | 249 | H | 6.5 | 4–23–48 | 440.2 | ..... |
| | 253 | H | 6 | 5–20–48 | 377.2 | 11– 4–48 |
| Av. | | | | | 317.8 | |
| | 2123 | J | 2.5 | 3–31–48 | 202.4 | 12– 6–48 |
| | 3902 | G | 4 | 6–26–48 | 343.1 | 11–26–48 |
| | 264 | H | 5 | 5–23–48 | 443.8 | 8– 7–48 |
| 4 | 300[a] | H | 2.5 | 4– 1–48 | 276.0 | ..... |
| | 212[b] | H | 8.5 | 6– 4–48 | 293.3 | 11–15–48 |
| | 231 | H | 7.5 | 5–21–48 | 398.6 | 8–12–48 |
| Av.[c] | | | | | 336.2 | |

[a] Removed from experiment at end of first period.
[b] Placed on experiment beginning of second period.
[c] Cows under footnote (a) not included in the group average.

*Rations.* The roughages used consisted of good quality, chopped alfalfa hay and a grass-legume silage. The dehydrated grass was a mixture of orchard grass, ladino clover, alsike clover and timothy. This mixture was seeded 2 yr. previous to dehydration on land which had been treated with 2 tons of limestone and 100 lb. of treble superphosphate per acre. The grasses were harvested at an average height of 10 in. and dehydrated in an Arnold Dehydrator at approximately 1,700° F. This material was fed in finely ground form.

The grain mixture used consisted of 200 lb. millrun, 400 lb. ground cull peas, 600 lb. ground oats, 300 lb. ground barley, 200 lb. ground whole corn, 300 lb.

linseed oil meal, 20 lb. iodized salt, 20 lb. steam bone meal, 4 oz. type 9-F irradiated yeast and 2 oz. of manganese sulfate, feeding grade. The average analyses of feeds as fed are shown in table 2.

*Procedure.* Silage was fed at the rate of 3 lb. for each 100 lb. of body weight. Grain was fed at the rate of 1 lb. for each 3 lb. of 4 per cent fat-corrected milk produced daily. The amount of silage and grain fed was adjusted weekly ac-

TABLE 2

*Analyses of feeds on a moisture basis as fed*

| | Alfalfa hay[a] | Silage[b] | Grain[c] | Dehydrated grass[d] |
|---|---|---|---|---|
| | (%) | (%) | (%) | (%) |
| Crude protein | 14.0 | 3.8 | 16.8 | 14.7 |
| Ether extract | 1.7 | 1.1 | 3.6 | 3.9 |
| Ash | 8.3 | 3.4 | 5.5 | 6.5 |
| Crude fiber | 34.7 | 13.1 | 9.3 | 21.4 |
| Nitrogen free extract | 32.9 | 13.1 | 55.6 | 44.4 |
| Dry matter | 91.5 | 34.5 | 90.8 | 90.8 |
| Total digestible nutrients | 49.1[e] | 20.3[f] | 71.3[f] | 66.8[e] |

[a] Av. of 8 samples
[b] Av. of 6 samples.
[c] Av. of 2 samples.
[d] Av. of 4 samples.
[e] Determined by digestion trial using 3 sheep.
[f] Calculated using Morrison's tables. (8)

cording to body weight changes and milk production. Hay was fed *ad libitum.* Dehydrated grass legume (hereafter referred to as dehydrated grass) was used to replace grain at three different levels. The four rations fed were as follows: (a) control—no grain replacement; (b) 15 per cent of grain replaced with dehydrated grass; (c) 30 per cent of grain replaced with dehydrated grass; (d) 45 per cent of grain replaced with dehydrated grass. These were straight

TABLE 3

*Design of the experiment*

| Ration | July 10–Aug. 21 | Aug. 28–Oct. 9 | Oct. 16–Nov. 27 | Dec. 4–Jan. 15 |
|---|---|---|---|---|
| | Period 1 | Period 2 | Period 3 | Period 4 |
| Control | Group 1 | Group 4 | Group 3 | Group 2 |
| 15% of grain replaced | Group 2 | Group 1 | Group 4 | Group 3 |
| 30% of grain replaced | Group 3 | Group 2 | Group 1 | Group 4 |
| 45% of grain replaced | Group 4 | Group 3 | Group 2 | Group 1 |

weight replacements. The cows were housed in a conventional tie-stall barn and were allowed approximately 2.5 hr. exercise per day in a paved yard. The mangers were equipped with metal partitions so that the feed intake of each cow was recorded accurately. All refused feed was collected and weighed. Composite samples of weighbacks for each cow were accumulated over each experimental period and analyzed.

As shown in table 3, the feeding trial was divided into four 6-wk. periods with a 1-wk. transition period between each change-over. The experiment ran a total of 28 wk. from July 3, 1948, to January 15, 1949. All cows were milked three times daily at regular 8-hr. intervals. Grain, dehydrated grass and hay were fed three times daily and silage twice daily. Feed analyses were made according to procedures outlined by the Association of Official Agricultural Chemists, sixth edition (1).

Feed consumption in terms of total digestible nutrients was calculated for each cow by weeks, using the results of digestion trials for the alfalfa hay and dehydrated grass and using digestion coefficients listed in Morrison's tables (8) for the grain and silage. Since moisture in weighbacks varied from cow to cow, it was necessary to take one sample per cow per period and determine the difference in weight of the sample at collection time as compared with the weight when the sample was allowed to air dry. The weighbacks for each cow were corrected accordingly. The total digestible nutrients then were calculated from an assumed ration of two-thirds hay and one-third silage, using Morrison's (8) digestion coefficients for hay and silage as applied to the chemical analyses as determined. Statistical analyses were made essentially as outlined in Snedecor (10).

The average chemical analyses and standard errors of weighbacks by rations are shown in table 4. There were no appreciable differences in average analyses

TABLE 4

*Average chemical analyses and standard error of weighbacks, by rations on a moisture per sample basis*

| | Rations | | | |
|---|---|---|---|---|
| | Amount of grain replaced with dehydrated grass | | | |
| | 0%[a] | 15%[b] | 30%[a] | 45%[b] |
| | (%) | (%) | (%) | (%) |
| Dry matter | 92.7 ± 0.48 | 93.2 ± 0.36 | 93.3 ± 0.32 | 91.4 ± 0.30 |
| Crude protein | 10.5 ± 0.31 | 10.5 ± 0.34 | 10.7 ± 0.88 | 12.0 ± 0.37 |
| Ether extract | 1.7 ± 0.10 | 1.6 ± 0.06 | 1.6 ± 0.07 | 1.7 ± 0.09 |
| Ash | 8.2 ± 0.19 | 8.0 ± 0.19 | 8.0 ± 0.20 | 8.0 ± 0.70 |
| Crude fiber | 40.9 ± 1.24 | 41.1 ± 1.82 | 40.9 ± 0.66 | 39.5 ± 0.87 |
| Nitrogen free extract | 31.5 ± 0.43 | 32.2 ± 0.37 | 32.1 ± 0.53 | 32.9 ± 0.48 |
| Estimated total digestible nutrients | 50.1 ± 0.42 | 50.6 ± 0.25 | 50.6 ± 0.33 | 50.8 ± 0.27 |

[a] 20 analyses
[b] 19 analyses

of weighbacks between groups, with the exception that crude protein was approximately 1.5 per cent higher in the weighbacks of cows on the 45 per cent ration. Since weighbacks were mostly all hay and some silage, the significance of this difference is not understood. Using these average analyses shown in table 4, the total digestible nutrient intake by cows by weeks was calculated. This was compared with maximum requirements for weight and production as outlined in Morrison's tables (8). The results of this study are shown in table 5. The cows, when fed the control ration, gained an average of 25.7 lb.

per 6-wk. period, as compared with 16.8, 18.6 and 8.5 lb. when they had 15, 30 and 45 per cent of their grain replaced with dehydrated grass, respectively. These differences in body weight gains between rations were significant as shown by the analysis of variance. The mean gain per week for the control was significantly higher than the mean gain for the 45 per cent ration. The other mean differences were not significant. The analysis of variance further revealed a

TABLE 5

*Milk produced, changes in body weight, total digestible nutrients consumed, maximum requirements (Morrison, 8) and amount consumed over maximum requirements*

| | Rations | | | |
|---|---|---|---|---|
| | Amount of grain replaced with dehydrated grass | | | |
| | 0%[a] | 15%[b] | 30%[a] | 45%[b] |
| | (*lb.*) | (*lb.*) | (*lb.*) | (*lb.*) |
| **Total digestible nutrients consumed** | | | | |
| Av./cow/week | 169.5 | 165.1 | 164.7 | 164.9 |
| **Maximum requirements** | | | | |
| Av./cow/week | 147.1 | 142.6 | 143.0 | 143.2 |
| **In excess of maximum requirements** | | | | |
| Av./cow/week | 22.4 ± 1.15 | 22.5 ± 1.12 | 21.7 ± 1.25 | 21.6 ± 1.45 |
| **Gains in body weight** | | | | |
| Total | | | | |
| Group 1[c] | 195.0 | 116.5 | 45.5 | 11.0 |
| Group 2 | 82.5 | 124.0[d] | 93.5 | 25.5 |
| Group 3 | 13.0 | 91.0 | 144.0 | 47.0 |
| Group 4 | 223.0 | 12.5 | 88.5 | 120.5[d] |
| Total | 513.5 | 319.0 | 371.5 | 182.0 |
| Av./cow/period | 25.7 ± 5.2 | 16.8 ± 5.3 | 18.6 ± 2.9 | 8.5 ± 5.7 |
| Av./cow/week | 4.3 | 2.8 | 3.1 | 1.6 |
| **4% fat-corrected milk produced** | | | | |
| Total | | | | |
| Group 1[c] | 8927.4 | 8739.8 | 7225.2 | 5896.5 |
| Group 2 | 5888.1 | 6155.5[d] | 8044.3 | 6733.2 |
| Group 3 | 7372.4 | 6228.2 | 8588.8 | 8474.5 |
| Group 4 | 8743.1 | 7196.6 | 5824.6 | 7379.9[d] |
| Total | 30931.0 | 28320.1 | 29682.9 | 28484.1 |
| Av./cow/week | 257.8 | 248.4 | 247.4 | 249.9 |

[a] 20 cows
[b] 19 cows
[c] 5 cows/group except where noted
[d] 4 cows

highly significant difference between periods of the trial. The winter of 1948–49 was extremely rigorous during November, December and January. The cows consumed more total digestible nutrients over maximum requirements and gained less during the periods beginning October 16 and December 4 than was the case with the first two periods. Since during this experiment there was a group of cows on each ration each period, this observed difference hardly can be ascribed to the rations. As further shown in table 5, the cows, when fed the 45 per cent grain replacement ration, varied more in weight gains per cow, as evidenced by a comparison of the standard errors. The coefficients of variability

were found to be 90.4, 139.4, 171.0 and 289.6 per cent for the control 15, 30 and 45 per cent rations, respectively. All of these coefficients of variability are high, but period differences attributed to thermal changes also enter in, as was shown earlier from the analysis of variance. A comparison of weight gains with intake of total digestible nutrients above minimum requirements showed negative correlations of –0.254, –0.367, –0.183 and –0.315 for the control 15, 30 and 45 per cent rations, respectively. These correlations are unusual, but true evaluation of thermal stress is yet to be determined. It is known that animals show increased appetite during cold weather, increased thyroxin output and possibly other physiological adaptations, as outlined by Lee and Phillips (7). The least weight gain and the greatest feed intake over minimum requirements occurred during the October 16 to December 4 period, which coincides with the rather sudden change in thermal conditions which occurred during the fall of 1948.

The production of 4 per cent fat-corrected milk also is shown in table 5 by groups and by rations. Cows on the control ration produced an average of 36.8 lb. of 4 per cent fat-corrected milk daily, as compared wtih 35.5, 35.3 and 35.7 lb. for the 15, 30 and 45 per cent rations, respectively. The superiority of the control ration for milk production, as compared with the other rations, was further shown by analysis of variance to be statistically significant. The lowered production obtained from the cows receiving dehydrated grass cannot be attributed to the consumption of insufficient total digestible nutrients, since the average daily consumption for these groups was from 3.1 to 3.2 lb. higher than Morrison's (8) maximum standards, as compared with 3.2 lb. daily excess for the cows when on the control ration.

While the total digestible nutrient value of the dehydrated grass is relatively high, thus causing little difference in total digestible nutrient consumption whether on the control, 15, 30 or 45 per cent rations, the crude fiber content of this material was 21.4 per cent, compared with 9.3 for grain. The heat increment necessary to digest the dehydrated grass must be proportionately greater than that of the concentrate mix, thus lowering the amount of energy available for productive purposes, *i.e.*, milk production and deposition of body fat.

Furthermore, the crude fiber content of the grain ration was increased noticeably when dehydrated grass replaced part of the grain. The crude fiber content of the grain ration was 9.3 per cent, as compared with 11.1, 12.9 and 14.8 per cent when 15, 30 and 45 per cent, respectively, of the grain ration was replaced by dehydrated grass. This additional crude fiber may have served to depress somewhat the digestibility of other ration constituents, as indicated by Swift *et al.* (11).

The reduced net energy and increased crude fiber concepts do not seem to explain why there was little difference in the production of 4 per cent fat-corrected milk between the three groups which had a portion of their grain replaced with dehydrated grass, regardless of whether the replacement was a 15, 30 or 45 per cent level. However, as previously pointed out, the cows on the 45 per cent ration gained less. Furthermore, average daily hay consumption varied little regardless of ration, since cows while on the control ration consumed 16.6

lb. of hay daily, as compared with 16.5 lb. for each of the grain replacement rations.

The physical state of the dehydrated grass was undesirable and may be a contributing factor.. Several cows showed impaired rumination when 30 per cent of their grain was replaced with dehydrated grass. Three cows were treated for rumen atony while on the 45 per cent ration. Also, additional trouble was experienced from anorexia in varying degrees of severity. These troubles were not evident when these same cows were on the control and 15 per cent rations. These observations are in agreement with those of Cole and Mead (3) and Watson and Ferguson (14). Of course, cows off feed lost weight and dropped off sharply in milk production. Upon recovery, milk flow increased again, but seldom to levels observed prior to digestive disturbances. The rate of lactation decline was least when the cows were on the control ration and greatest when 45 per cent of the grain ration was replaced with dehydrated grass. These differences are clearly shown in figure 1. While the rate of lactation decline in-

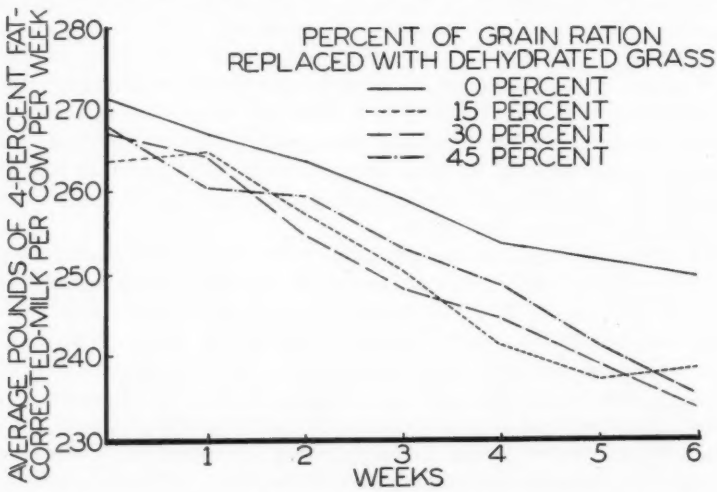


FIG. 1. Average lactation decline by rations for 6-wk. experimental periods.

creased during the fourth, fifth and sixth weeks for the 30 per cent and 45 per cent rations, as compared with the controls, this rate only approached significance ($t.05 = \pm 17.58$ lb.; mean difference between 30 per cent ration and control was 16.0 lb.).

The amount of total digestible nutrients required for maintenance and the production of 100 lb. of 4 per cent fat-corrected milk was 59.9, 62.5, 62.2 and 63.7 lb. for the control, 15 per cent, 30 per cent and 45 per cent rations, respectively. These calculations were made by deducting 3.53 lb. of total digestible nutrients per pound of body gain according to data reported by Knott (6). This does not take in account those instances where cows lost weight during a

portion of, and in some cases during all of, an experimental period. Technically this might alter the results shown above, but it is not considered good dairy cattle management to have cows in mid-lactation lose weight. For practical purposes, the above levels of efficiency would be of importance. Direct comparison of this experiment with other published experiments is not feasible, since the dehydrated grass was a high quality commercial product lower in protein and showed less total digestible nutrients (66.8 per cent digestible) than the materials used in earlier experiments (2, 5, 9, 12, 14). Furthermore, the experimental periods in this trial were 6 wk. in length as compared with 2 wk. for Watson (12) and Knott *et al.* (5), 5 wk. for Watson and Ferguson (14) and 3 wk. for Camburn (2). Newlander (9) replaced all the concentrates with dehydrated grass for 6 wk. and observed a 9.9 per cent decline in milk yield as compared with controls. The average production of cows in this experiment was slightly greater than 35 lb. of 4 per cent fat-corrected milk for the entire 28 wk. experimental period. Several cows were milking in excess of 60 lb. of 4 per cent fat-corrected milk at the start of the experiment.

## SUMMARY

Twenty high producing dairy cows were divided into four experimental groups consisting of three Holsteins, one Jersey and one Guernsey per group. Three rates of grain replacement (15, 30 and 45 per cent) with a commercially dehydrated grass-legume mixture were studied a total of 28 wk., using 6-wk. experimental periods and 1 wk. for transition for each group of cows for each ration. The following results were observed:

(a) Cows, when fed the control ration, gained an average of 25.7 lb. in body weight per cow per period, as compared with 16.8, 18.6 and 8.5 lb. when they had 15, 30 and 45 per cent, respectively, of their grain replaced with dehydrated grass.

(b) A digestion trial with three sheep showed that the commercially dehydrated material used contained 66.8 per cent total digestible nutrients on an air-dry basis.

(c) Cows fed the control ration produced an average of 36.8 lb. of 4 per cent fat-corrected milk daily, compared with 35.5, 35.3 and 35.7 lb. when they had 15, 30 and 45 per cent of their ration replaced with dehydrated grass, respectively. This difference in production in favor of the control ration was statistically significant.

(d) The finely ground physical state of the dehydrated grass-legume mixture was undesirable, since cows having 30 and 45 per cent of their grain replaced showed varying degrees of rumen atony and anorexia. This condition was not observed when the cows were on the control and 15 per cent rations.

(e) Cows receiving no dehydrated grass required less total digestible nutrients for maintenance and production of 100 lb. of 4 per cent fat-corrected milk than when they received dehydrated grass.

(f) The rate of decline in milk yield was less rapid when the cows were fed the control ration, but these mean differences only approached significance.

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# THE EFFECT OF VITAMIN A FROM PRENATAL STORAGE AND FROM INGESTION OF COLOSTRUM ON THE NEONATAL CALF[1]

L. NEZVESKY,[2] H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, C. I. BLISS AND A. A. SPIELMAN

*Animal Industries Department, Storrs Agricultural Experiment Station, University of Connecticut, Storrs*

The effect of prenatal nutrition on the subsequent performance of the newborn recently has received considerable attention. Both the prenatal storage and colostral content of vitamin A can be increased in the ruminant by dietary means (10, 13, 16, 18, 20, 21). These two sources of vitamin A have been demonstrated in independent work to be available to the neonatal calf (4, 7). In combination, they have been held to be responsible for increases in the levels of plasma vitamin A of neonatal calves and lambs and in superior performance of neonatal Holstein calves, as indicated by greater weight gains and lower incidence of scours (3, 17). With one exception, Fountaine *et al.* (10), the increases followed the prepartum feeding of supplementary vitamin A *per se*. The number of days of feeding the prepartum supplement has varied widely among workers and in experiments by the same worker. The relative contributions of vitamin A to the neonatal calf from prenatal storage and from colostrum were not determined.

Since vitamin A is relatively expensive (the feeding of one million units daily for 30 days prepartum would cost approximately three dollars per cow at present prices), experiments are needed to determine the minimum level and the best feeding schedule for prepartum supplements of vitamin A. However, to establish the optimum performance of the neonatal calf, information is needed as to the relative contribution of vitamin A from prenatal storage and from colostrum. This was the objective of the present study, which has been based upon measurements of total blood hemoglobin, carotene and vitamin A in the plasma and liver, whole blood and plasma ascorbic acid, liveweight, fecal pH and dry matter and incidence and duration of scours.

## EXPERIMENTAL

*Animals.* Twenty-eight cows of the Ayrshire, Guernsey, Holstein and Jersey breeds which had calved in the University of Connecticut herd from November, 1948, through May, 1949, were used in the experiment. One half of these cows received a basal ration for 8 wk. prior to the calculated parturition date, and

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[2] Present address: College of Veterinary Medicine, Cornell University, Ithaca, New York.

the other half received the same basal ration plus one million U.S.P. units of vitamin A daily for 30 days prior to the calculated parturition date. The levels of feeding and the composition of the feeds and supplementary vitamin A[3] are essentially as described previously (5).

The calves born to these cows were not allowed to nurse but were removed to individual pens in the calf barn. There they received colostrum from frozen pooled colostrum banks. The allotment of calves to dietary groups was as follows:

| Dams | Colostrum | |
|---|---|---|
| | From Basal dams | From basal + vitamin A dams |
| Basal dams | Group I | Group II |
| Basal + vitamin A dams | Group III | Group IV |

Four separate colostrum banks of basal colostrum and a similar number of basal plus vitamin A colostrum were used and represented the first two milkings after parturition. The first bank was from cows fed rations identical to those in this experiment and was made up immediately prior to the birth of the first calf on this experiment. The second and following banks were made up from cows on the experiment, as the prospective need for additional colostrum became evident, in order to have a supply of colostrum on hand at all times. In the basal colostrum banks, the carotene averaged 278 γ per cent, ranging from 249 to 309 γ per cent, and the vitamin A, 273 γ per cent with a range of 166 to 347 γ per cent. Similar values for the basal plus vitamin A banks were carotene, 216 γ per cent with a range of 174 to 309 and vitamin A 706 γ per cent with a range of 491 to 1217.

The newborn calves received four feedings of colostrum in the first 2 days at the rate per feeding of 3.75 lb. to Ayrshires and Holsteins and 3 lb. to Guernseys and Jerseys. They then were fed herd milk, starter and U. S. no. 2 clover-timothy mixed hay according to the Cornell drycalf starter method (19). Both colostrum and milk were fed in nipple pails. Water was allowed *ad lib.* after the first week. The temperature in the calf barn was maintained at a minimum of 16° C. by radiant heat from the ceiling.

*Samples, observations and analyses.* Venous blood samples were collected immediately after birth, before the second, third, fifth, seventh and tenth feedings of colostrum or herd milk and at 7, 10, 14, 21 and 28 days of age. Total hemoglobin, plasma carotene and vitamin A, and whole blood and plasma ascorbic acid were determined with an Evelyn photoelectric macro-colorimeter by the methods of Evelyn and Malloy (8), Kimble (12), and Roe and Kuether (14), respectively. Calves were weighed at birth and at 4-day intervals thereafter to 28 days of age. Daily fecal samples were obtained until the calf was 15 days of age and, in addition, at 18, 21 and 28 days, by rotating a clinical thermometer

[3] The vitamin A supplement, shark liver oil containing 25 per cent by weight of crude soybean lecithin, was generously supplied by the Nopco Chemical Co., Harrison, N. J.

in the anus of the calf. Fecal pH was determined by the method of Shoskes (15) and dry matter by oven-drying at 100° C. for 24 hr. Four calves each from groups I and III, three from group IV and two from group II were slaughtered at the end of the 28-day experimental period and liver carotene and vitamin A determined by the method of Davies (1).

Standard statistical procedures as outlined by Davies (2) and Fisher and Yates (9) were used to test for differences between treatments. In addition, the liveweight data were analyzed according to the methods proposed by Wishart (23).

## RESULTS AND DISCUSSION

*Hemoglobin.* Total hemoglobin did not vary significantly between treatments, but decreased with age. Since this trend was in accord with the report of Wise *et al.* (22), the data need not be cited.

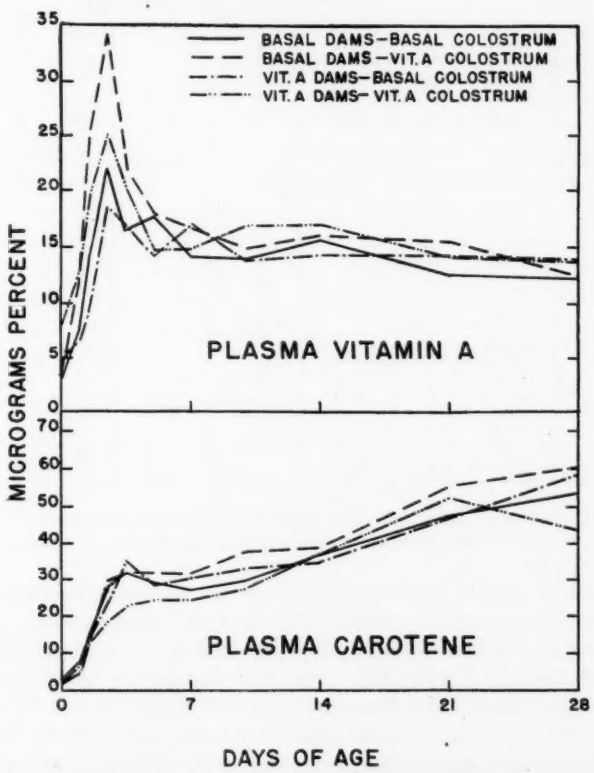


FIG. 1. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the blood plasma levels of carotene and vitamin A in the young calf.

*Carotene and vitamin A.* The levels of plasma carotene (fig. 1) were not affected significantly by treatment. The vitamin A content of the plasma col-

lected for the first 5 days after birth was significantly higher ($P < 0.001$) in both groups of calves fed colostrum from dams receiving prepartum supplements of vitamin A. In later samples from the 7th to the 28th day the difference was in the same direction, though not significant. The plasma level of vitamin A at birth was significantly higher ($P < 0.01$) in calves from dams fed prepartum supplements of vitamin A than that in calves from dams on the basal ration alone.

The liver storage of carotene at 28 days of age (fig. 2) was decreased, but

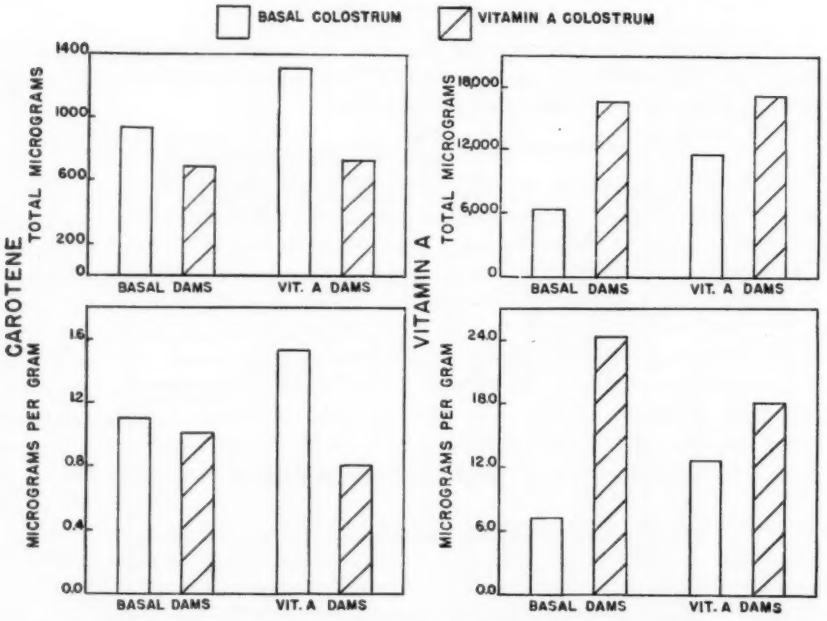


FIG. 2. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the geometric mean content of carotene and vitamin A in the liver of calves at 28 d. of age.

not significantly, by the feeding of colostrum from dams receiving prepartum supplements of vitamin A, but practically unaffected by prenatal storage. The liver storage of vitamin A at 28 days of age was increased markedly ($P < 0.01$ per gram of liver and $P < 0.05$ for total liver storage) by the feeding of colostrum from dams fed the supplementary vitamin A. Although the additive influence of prenatal storage on the vitamin A content of the liver was not of statistical significance, an analysis of the liver stores at 28 days in the two groups of calves receiving the basal colostrum showed a nearly significant increase due to the influence of the prepartal supplements of vitamin A. Prepartum supplements of vitamin A increased the level of vitamin A in the neonatal calf, primarily through the colostrum. High levels of vitamin A possibly may be pro-

duced by feeding vitamin A for shorter periods than was the case in this work or in similar studies as reviewed by Parrish *et al.* (13).

*Ascorbic acid.* Neither whole blood nor plasma ascorbic acid (fig. 3) was affected significantly by treatment. The ascorbic acid levels decreased markedly immediately after birth and then continued to fall at a slower rate. These results are essentially in agreement with those of Hibbs and Krauss (11), both

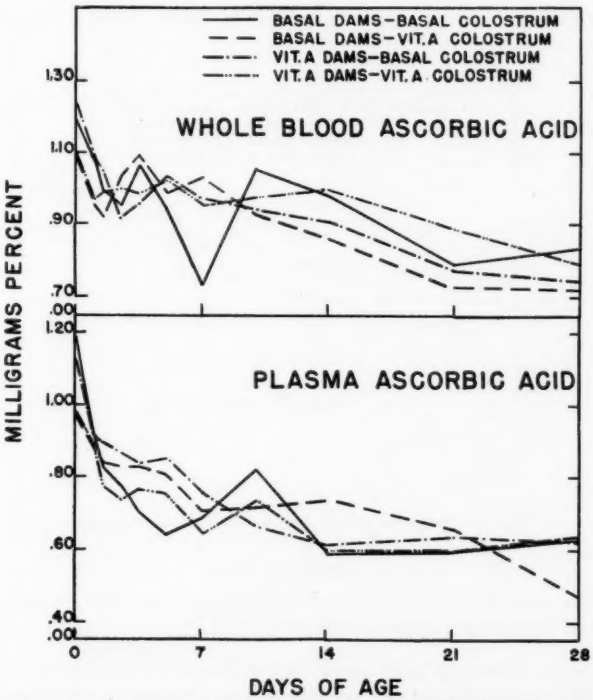


FIG. 3. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the whole blood and plasma ascorbic acid levels in the young calf.

in trend and in the lack of correlation between the plasma and liver level of vitamin A and plasma ascorbic acid in the neonatal dairy calf.

An analysis of all observations for whole blood ascorbic acid and for plasma ascorbic acid showed significant differences ($P < 0.001$) between calves and between ages; however, the level of significance for the plasma ascorbic acid, as indicated by the $F$-value, was greater than that for whole blood. In contrast, calculated cellular ascorbic acid, uncorrected for cellular volume, did not show significant differences between calves and gave a lower level of significance ($P < 0.01$) between ages. Therefore, plasma ascorbic acid is a more sensitive criterion of the ascorbic acid level of blood because of the relative stable cellular ascorbic acid content.

*Liveweight.* An analysis of the changes in liveweight (fig. 4), in terms of either total gain or rate of increase (23), with or without adjustment for weight at birth, revealed no significant differences between treatments. Since the number of calves was small and represented four breeds, one could expect only wide differences to be detectable.

*Fecal pH and dry matter and incidence of scours.* Neither fecal pH nor fecal dry matter (fig. 5) showed a significant difference between treatments, but both varied with increasing age. The cause of these changes has yet to be determined.

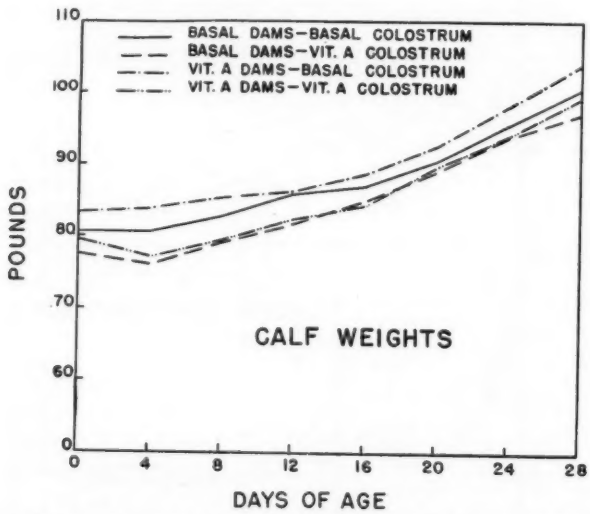


FIG. 4. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the liveweight changes in the young dairy calf.

Five cases of relatively mild scours were observed, but, contrary to expectation, they were accompanied by no appreciable change in the content of fecal dry matter or pH. No group had more than two cases and none showed any relation to vitamin A treatment.

## SUMMARY

The effect of vitamin A from prenatal storage and from ingestion of colostrum was studied from birth to 28 days of age in 28 dairy calves. One half of these calves were from dams receiving only a basal ration and one half were from dams receiving the same basal ration plus one million U.S.P. units of vitamin A daily for 30 days prior to the calculated date of parturition. At birth the calves from each of the maternal dietary groupings were subdivided into two groups, one receiving colostrum from dams fed only the basal ration and the other receiving colostrum from dams fed the basal ration plus the supplementary vitamin A.

The data indicate that colostrum significantly increased the plasma vitamin

A from birth to 5 days of age and liver vitamin A at 28 days of age. Prenatal storage elevated the blood plasma vitamin A level at birth significantly and contributed to greater liver storage of vitamin A at 28 days of age. The

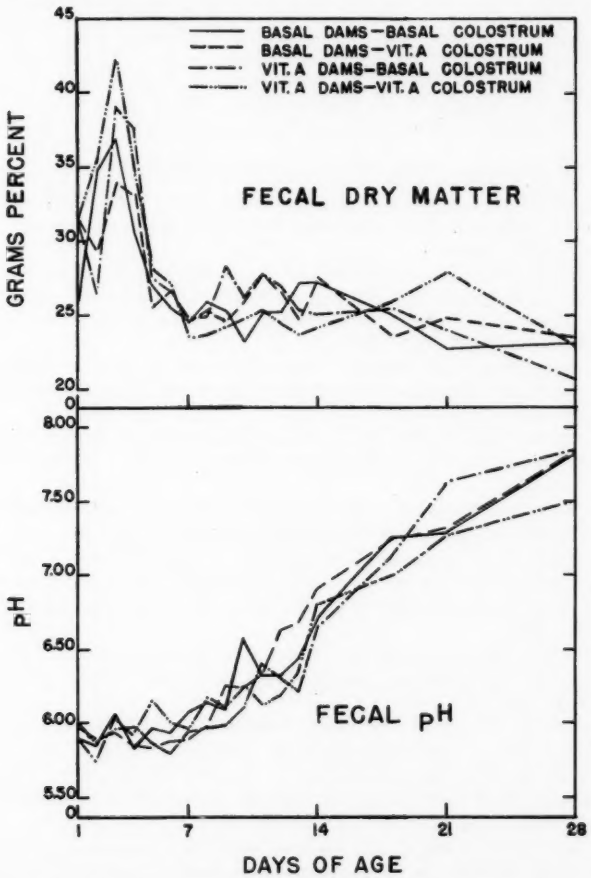


FIG. 5. The effect of vitamin A from prenatal storage and from ingestion of colostrum on the fecal pH and dry matter in the young dairy calf.

other criteria measured, hemoblobin, whole blood and plasma ascorbic acid, liveweight, fecal pH and dry matter and incidence of scours, were not affected significantly by treatment.

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# STUDIES OF HEATED MILK. I. FORMATION OF 5-HYDROXYMETHYL-2-FURFURAL[1]

STUART PATTON

*Department of Dairy Husbandry, Pennsylvania Agricultural Experiment Station, State College, Pa.*

Some recent research has focused interest on furan compounds and their possible relation to the so-called browning reaction. Singh *et al.* (7) have shown that the darkening of sugar solutions is related to the formation of hydroxymethylfurfural and levulinic acid. Patton and Josephson (5) have isolated furfuryl alcohol from skim milk which had undergone browning as a result of heat treatment. The compound maltol, an isomer of hydroxymethylfurfural, also has been isolated from heated skim milk (4). The importance of furan compounds has been firmly established in the browning of apricot concentrates (1).

Fundamental investigations of the browning reaction by Wolfrom *et al.* (8) have revealed that hydroxymethylfurfural is formed by heating aqueous systems of glucose and glycine. These workers also report that hydroxymethylfurfural, in sufficient concentration, forms deeply colored products with glycine and at a high rate of speed.

It is well known that certain dairy products undergo browning rather readily as a result of heat treatment, storage at conducive temperatures or both. Of the various milk constituents, lactose and casein are known to be intimately concerned in the color change (2).

These interesting observations from the literature have suggested the possibility that hydroxymethylfurfural may be a concomitant of browning in heated milk. The present paper reports a fundamental study of this point.

## EXPERIMENTAL

*Preparation of pure hydroxymethylfurfural.* In order to obtain known derivatives of hydroxymethylfurfural, the pure compound was prepared by the method of Middendorp (3) with slight modifications. Sucrose (200 g.) and oxalic acid hydrate (1.4 g.) were dissolved in 600 ml. of water. The solution was heated in an autoclave at 127° C. for 2.5 hr. After cooling to room temperature, the reaction mixture was neutralized with $CaCO_3$ and then gently agitated with 10 g. basic lead acetate for 1 hr. Suspended material was removed from the mixture by centrifuging. The clear supernatant containing the hydroxymethylfurfural was extracted with ethyl acetate for 10 hr. in a continuous extraction unit. The ethyl acetate extract was dried, the solvent removed and the crude hydroxymethylfurfural purified by distillation under high vacuum; yield 14 g. m.p. 32–33° C.

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The following derivatives, with melting points as indicated, were prepared from the purified hydroxymethylfurfural: semi-carbazone (194–195° C.), phenylhydrazone (140–141° C.) and 5-hydroxymethyl-2-furoic acid (165–166° C.). The melting points of these derivatives were in accordance with values reported for them in the literature (6).

*The interaction of lactose and glycine.* In view of the findings by Wolfrom *et al.* (8) that hydroxymethylfurfural is formed by the heating of aqueous glucose-glycine systems, it was considered of interest to study the character of this reaction as applied to lactose. Glycine, having amino and carboxyl groups, should present a simplified version of similar reactive groupings in a protein particle.

β-Lactose (C.P., 150 g.) and glycine (30 g.) were dissolved in 820 ml. of water. This solution (pH 7.0) was autoclaved for 2.5 hr. at 127° C., after which it was cooled to room temperature, neutralized with a 10 per cent suspension of $Ca(OH)_2$ and centrifuged. The clear, dark-brown supernatant was extracted with ether for 24 hr. in a continuous extraction unit. The ether extract was dried and, after evaporation of the solvent, a small quantity (0.4 g.) of light brown oil remained. This oil, upon distillation under high vacuum ($< 0.5$ mm.), proved to be fairly pure hydroxymethylfurfural. The distillate solidified readily in the cold and, on subsequent warming, melted at 32–33° C. It had a bitter, acrid taste and gave an intense purple color in the Molisch test. The semicarbazone and phenylhydrazone derivatives were prepared readily from it and found to melt at 194–195° and 140–141° C., respectively. These derivatives showed no melting point depression on admixture with authentic samples of like melting point.

A similar experiment, in which the only variation was to increase the time of autoclaving to 5 hr., yielded 1.28 g. of crude hydroxymethylfurfural. Small quantities of maltol were formed in these lactose-glycine systems (4), but no furfuryl alcohol could be detected.

*Carbohydrate-casein systems.* With the formation of hydroxymethylfurfural in heated lactose-glycine systems established, it was decided to ascertain the effect of replacing glycine with casein on this reaction. A sodium caseinate sol was prepared as follows: Raw skim milk was coagulated by the addition of 1 *N* HCl. After the curd had settled, the whey was drained and the curd then washed four times, under gentle agitation, with volumes of water equivalent to the whey removed. Resuspension of the acid casein was accomplished by taking it up in water, adjusting the pH to 6.7 with *N*/3 NaOH and agitating the sol for several hours at 20–25° C. The few gross particles remaining were removed by filtration of the sol through fine filter cloth. The total solids content of the sol was determined to be 6.65 per cent.

Lactose (150 g.) was dissolved in 850 ml. of the casein sol and the mixture autoclaved for 2.5 hr. at 127° C. This mixture, following autoclaving, was neutralized, clarified and extracted in the same fashion as the lactose-glycine system except that the heat-coagulated casein curd was pressed in an hydraulic press to obtain the maximum amount of "whey" for ether extraction. An extract

residue of 0.56 g. was obtained after removal of solvent. Vacuum distillation of this residue produced three fractions, the principle constituents of which were 270 mg. of furfuryl alcohol, 70 mg. of maltol (by sublimation), and 160 mg. of hydroxymethylfurfural.

The identification of furfuryl alcohol and maltol was accomplished by a study of their physical and chemical properties, the preparation of suitable derivatives and the performance of appropriate melting and mixed melting point determinations. These procedures have been described previously in detail (4, 5) and were attended meticulously in all experiments reported in this paper. Hydroxymethylfurfural was identified in the manner described for this compound from the lactose-glycine system. This method also was used in subsequent experiments reported herein.

Two additional systems were prepared with the casein sol. In these, lactose was replaced with equal weights of D-glucose and D-galactose (both C.P.). Autoclaving, refining, extraction, etc. of these systems were the same as for that of lactose. No detectable quantities of either furfuryl alcohol or maltol were found in the extract residues obtained in these experiments. The crude residues yielded 0.62 g. of hydroxymethylfurfural for the galactose system and 0.61 g. for that of glucose. These three experiments concerning lactose, glucose and galactose were repeated utilizing a 7.5 per cent aqueous suspension of commercial casein (Arthur H. Thomas Co.) in place of the casein sol. The results were very similar in all respects to those obtained when the casein sol was employed.

*Heated skim milk.* In these experiments, condensed skim milk (30 per cent total solids) was used. The use of this product appeared to have several advantages over using plain skim milk. The reactants in the condensed skim milk are more concentrated, thus less milk need be handled. Further, it was found that better yields of the end-products are obtained when condensed skim milk is used, although qualitative tests showed that the same compounds are produced by heating plain skim milk. The use of whole milk was avoided, since milk fat would have complicated the extraction and purification procedures considerably. It seems unlikely that the presence of milk fat would have had any great bearing on the matter under investigation.

Several very similar experiments were conducted, of which the following is representative. Six liters of condensed skim milk were autoclaved for 2.5 hr. at 127° C. and then cooled to room temperature. The whey (3600 ml.), obtained by decantation from and expression of the heat coagulated curd, was neutralized, centrifuged and extracted with ether as described for the simplified systems. The solvent was evaporated, the final trace under vacuum, and the extract residue (3.0 g.) washed with two 15-ml. portions of petroleum ether to remove lipid material (0.50 g.). The residue was distilled under high vacuum and the following fractions obtained: Low boiling, unidentified, 0.37 g.; furfuryl alcohol, 1.25 g.; maltol (by sublimation), 0.42 g.; hydroxymethylfurfural, 0.11 g.; non-distilling residue, 0.20 g.

The rather disproportionately large amounts of non-distilling residue obtained in the skim milk extraction experiments as compared with those of the

simplified system created a problem. It appeared that weakly acidic substances in the residue were promoting polymerization which slightly reduced the yield and purity of the hydroxymethylfurfural fraction. However, the semicarbazone and phenylhydrazone derivatives were satisfactorily prepared from it and the chemical and physical properties of the fraction were found to be in good agreement with those of hydroxymethylfurfural.

*Control experiments.* One liter of aqueous, 15 per cent lactose solution was autoclaved at 127° C. for 2.5 hr. As a result of autoclaving, this solution showed little discoloration, whereas, the reaction mixtures, from which hydroxymethylfurfural was isolated, all were appreciably darkened in color by autoclaving. No measurable quantity of hydroxymethylfurfural could be recovered by ether extraction of the heated lactose solution.

The ether extraction procedure for recovering hydroxymethylfurfural from aqueous systems was found to be effective. Three grams of hydroxymethylfurfural, dissolved in 2 l. of water, was 93 per cent recovered by continuous extraction with ether for 24 hr.

## DISCUSSION

Unfortunately, there are no suitable quantitative technics for measuring hydroxymethylfurfural or furfuryl alcohol in a complex system such as heated milk. Thus, it was necessary to limit the scope of these experiments to compound isolation. As a consequence, no great stress can be placed upon the quantities of compounds isolated, since very small yields were obtained and some losses during isolation were inevitable. Therefore, where quantitative data have been presented, the primary purpose was to show that the compounds were recovered in some quantity in relatively pure form.

These experiments have demonstrated that hydroxymethylfurfural is formed during prolonged heating of concentrated skim milk. The recovery of the compound from heated lactose-glycine and lactose-casein systems has indicated that lactose is the origin. The results of the control experiment have shown that the conversion of lactose to small quantities of hydroxymethylfurfural is facilitated by the presence of glycine, casein or the heat degradation products of casein. Since it was observed that a pure lactose solution did not darken, whereas all the systems forming hydroxymethylfurfural browned readily, under the experimental conditions, it would appear that hydroxymethylfurfural formation is associated with browning in these systems. It is interesting to note that cleavage of the $\beta$-linkage of lactose is implied in its conversion to hydroxymethylfurfural. The experiments with glucose and galactose revealed that either hexose portion of the lactose molecule could give rise to the compound.

The compound which is recovered most readily and in highest yield from heated skim milk is furfuryl alcohol. Patton and Josephson (5) have suggested that either lactose or ascorbic acid might serve as the origin of furfuryl alcohol in heated skim milk. These experiments effectively preclude ascorbic acid as a possible origin, since yields of furfuryl alcohol exceeding that theoretically possible from ascorbic acid were obtained from heated skim milk. In addition, furfuryl alcohol was recovered from a lactose-casein system containing no ascorbic

acid. The mechanism of furfuryl alcohol formation from lactose is presently under investigation and will be the subject of a subsequent paper.


## SUMMARY

Hydroxymethylfurfural has been identified as one of the compounds formed during prolonged heat treatment of concentrated skim milk. From studies of simplified systems, it has been shown that this compound is formed from lactose. It further was demonstrated that either the glucose or galactose portion of the lactose molecule may serve as the origin of hydroxymethylfurfural. Glycine, casein or the heat degradation products of casein were found essential in the conversion of lactose to hydroxymethylfurfural. No measurable quantities of hydroxymethylfurfural were formed by heating lactose solutions alone under the experimental conditions employed. The formation of hydroxymethylfurfural appeared to be directly associated with heat-induced browning in these experiments.

In addition to hydroxymethylfurfural, furfuryl alcohol and maltol were formed during the heating of lactose-casein systems. Previous findings (4, 5) with regard to furfuryl alcohol and maltol formation in heated skim milk were confirmed.



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# PARTURIENT PARESIS. VI. SOME CHANGES IN THE URINARY EXCRETION OF CERTAIN CONSTITUENTS AT PARTURITION AND THEIR POSSIBLE ASSOCIATION WITH CHANGES IN THE BLOOD PICTURE[1, 2, 3]

T. H. BLOSSER

*Department of Dairy Husbandry, State College of Washington, Pullman*

AND

VEARL R. SMITH

*Department of Dairy Husbandry, University of Wisconsin, Madison*

Since the time when Little and Wright (16) first showed a marked diminution of blood calcium in cows with parturient paresis, many theories have been advanced to explain the blood changes which occur. Unfortunately, not many of the theories are supported by experimental evidence.

It is well known that certain hormones can alter the renal threshold for various blood constituents. For example, in hyperparathyroidism, according to Grollman (10), there is a lowered renal threshold for phosphorus. As a result, the blood phosphorus is lowered, and the urinary excretion of phosphorus is considerably increased. The parathyroids also exert some influence on the urinary excretion of calcium.

Shorr *et al.* (22, 23) demonstrated that certain of the steroidal sex hormones influence the urinary excretion of calcium and citric acid. There was no accompanying change in the blood composition.

In the expulsion of the placenta at the time of parturition, the animal loses the source of considerable hormone production. Following this loss, there undoubtedly is considerable interplay among the endocrine glands in readjusting to the changed conditions following parturition. It is not inconceivable that in this dynamic state of affairs the renal threshold for certain of the blood constituents might be altered or the urinary excretion changed by some other mechanism.

In addition to the above possibility, it also is possible that some sort of kidney disfunction might occur in connection with milk fever. In humans, renal disfunction frequently is associated with diseases occurring during pregnancy and at parturition. In eclampsia, for example, the toxemia responsible for the convulsive state may arise as the result of an improperly functioning kidney (1).

Several investigators (5, 8, 11, 12, 13, 18, 21) have studied the urine of nor-

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mal and milk-fever cows from a qualitative standpoint. No one has reported quantitative studies made in connection with the urinary excretion of the several constituents known to be involved in milk fever.

Since there is a paucity of information on some of the more basic changes which occur in milk fever and because of the possibility of kidney involvement, studies were initiated to determine quantitatively the urinary excretion of certain substances whose blood level is known to change during the course of the disease.

## EXPERIMENTAL PROCEDURE

Eight Jersey cows were used in the urinary excretion studies reported herein. Four of the cows were in the University of Wisconsin dairy herd and four were in the herd of the State College of Washington.

All cows in this experiment were handled in a conventional manner at, and following, parturition. With one exception, cows developing milk fever were treated by the injection of calcium gluconate. Cow B63 was treated by the udder inflation method.

Quantitative studies on urinary excretion necessitate collection of all urine excreted over a 24-hr. period. It soon was found that all urine could be collected with a minimum of labor by inducing micturition every 2 hr. according to the method developed by Turner (27).

At the end of a collection day, the 24-hr. specimen was measured, and an aliquot was taken to be used for subsequent chemical analysis. The aliquot was acidified and stored under toluene.

Attempts were made to collect 24-hr. urine specimens 10 and 5 days prepartum, and starting with the third day prepartum through parturition to the third day postpartum. Collections also were made 5 and 10 days postpartum. When cows with parturient paresis were treated by calcium therapy, no subsequent collections were taken during the first 4 days postpartum, because of the confusing effect which calcium gluconate has on the urinary excretion picture.

It is not possible to predict accurately the exact day of parturition. Consequently, the prepartum data do not always conform to the specific standards which were originally set, insofar as collection days are concerned.

Blood samples were drawn daily from the external jugular vein at about the middle of the collection day. With the milk fever cows, blood samples were drawn immediately prior to treatment, even if a sample had been drawn only a few hours previous.

The following methods were used in the analysis of blood: Blood serum calcium, method of Clark and Collip (4); blood serum magnesium, method of Simonsen *et al.* (24); blood plasma inorganic phosphorus, method of Fiske and Subbarow (9).

Urinary constituents were analyzed by the following methods: Calcium, method of Morris *et al.* (19); magnesium, method of Briggs (3); inorganic phosphorus, method of Tisdall (26).

Both blood serum and urinary citric acid determinations were made by the method of Perlman *et al.* (20) in the studies made at the University of Wiscon-

TABLE 1

*Average levels of certain blood constituents for specific periods of time previous and subsequent to parturition*

| Days prepartum | Calcium (*mg.* %) | | | Magnesium (*mg.* %) | | | Citric Acid (mg. %) | | | Inorganic phosphorus (*mg.* %) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normal cows | Milk-fever cows | Borderline cows | Normal cows | Milk-fever cows | Borderline cows | Normal cows | Milk-fever cows | Borderline cows | Normal cows | Milk-fever cows | Borderline cows |
| More than 5 d. | 10.9 | 11.4 | 10.9 | 2.30 | 2.43 | 2.45 | ..... | 4.93 | 6.77 | 5.50 | 3.12 | ..... |
| 5 and 4 d. | 10.5 | 10.8 | 10.2 | 2.30 | 2.19 | 2.36 | 4.33 | 5.05 | 4.52 | ..... | 4.02 | 4.08 |
| 3 and 2 d. | 10.4 | 11.5 | 10.4 | 2.19 | 2.39 | 2.41 | 5.31 | 6.20 | 5.28 | 6.48 | 4.26 | 5.38 |
| 1 d. | 10.0 | 10.6 | 10.6 | 2.42 | 2.33 | 2.50 | 5.12 | 9.12 | 6.61 | 6.61 | 3.94 | 5.39 |
| Day of parturition | 10.9 | 6.0 | 6.5 | 2.20 | 3.08 | 3.30 | 3.82 | 3.65 | 4.44 | 4.44 | 2.06 | 2.40 |
| Days postpartum | | | | | | | | | | | | |
| 1 d. | 9.2 | 5.8 | 7.0 | 2.45 | 3.74 | 3.38 | 4.70 | 3.53 | 2.65 | 3.96 | 2.61 | 3.75 |
| 2 and 3 d. | 10.0 | 6.8 | 8.4 | 2.30 | 3.25 | 2.78 | 5.12 | 1.74 | 5.00 | 4.46 | 3.43 | 4.77 |
| 4 and 5 d. | 11.5 | 10.5 | 10.2 | 2.34 | 1.88 | 2.02 | 5.63 | 4.19 | 3.30 | 5.36 | 4.77 | 3.44 |
| 10 d. | 11.1 | 11.0 | 11.0 | 2.30 | 1.96 | 1.79 | ..... | 6.18 | 4.78 | 5.73 | 5.44 | 4.19 |

TABLE 2

*Average 24-hour excretion of certain urinary constituents for specific periods of time previous and subsequent to parturition*

| Days prepartum | Calcium (*g.*) | | | Magnesium (*g.*) | | | Citric acid (*g.*) | | | Inorganic phosphorus (*g.*) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Normal cows | Milk-fever cows | Borderline cows | Normal cows | Milk-fever cows | Borderline cows | Normal cows | Milk-fever cows | Borderline cows | Normal cows | Milk-fever cows | Borderline cows |
| More than 5 d. | 0.157 | 0.973 | 0.142 | 1.851 | 4.646 | 2.869 | 0.421 | 1.621 | 0.591 | 0.0294 | 0.0266 | 0.0197 |
| 5 and 4 d. | 0.410 | 0.638 | 0.394 | 1.657 | 3.580 | 3.347 | 0.605 | 1.264 | 1.537 | 0.0176 | 0.0345 | 0.0339 |
| 3 and 2 d. | 0.333 | 0.129 | 0.206 | 1.391 | 2.822 | 2.961 | 0.700 | 2.959 | 0.990 | 0.0447 | 0.0231 | 0.0317 |
| 1 d. | 0.083 | 0.334 | 0.096 | 1.629 | 1.815 | 2.826 | 0.572 | 3.199 | 0.946 | 0.0514 | 0.0256 | 0.0252 |
| Day of parturition | 0.432 | 0.091 | 0.074 | 1.820 | 1.611 | 1.474 | 1.257 | 0.908 | 0.795 | 0.0778 | 0.0239 | 0.0534 |
| Days postpartum | | | | | | | | | | | | |
| 1 d. | 0.082 | 0.114 | 0.298 | 2.782 | 1.670 | 2.569 | 0.806 | 0.314 | 0.965 | 0.0622 | 0.0319 | 0.3416 |
| 2 and 3 d. | 0.256 | ..... | 0.232 | 2.991 | ..... | 2.667 | 0.869 | ..... | 0.915 | 0.557 | ..... | 3.5681 |
| 4 and 5 d. | 0.427 | 0.130 | 0.251 | 2.896 | 1.959 | 1.404 | 0.754 | 0.585 | 0.700 | 0.0305 | 0.8053 | 0.2233 |
| 10 d. | 0.191 | 0.239 | 0.336 | 3.328 | 2.755 | 2.393 | 1.355 | 0.901 | 1.188 | 0.0698 | 0.0734 | 0.6348 |

sin and by the method of Taussky and Shorr[4] (25) in studies made at the State College of Washington. The latter method was somewhat more sensitive and reproducible, but over-all results using the two methods were essentially the same.

## RESULTS AND DISCUSSION

Of the eight Jersey cows which were studied, four developed definite symptoms of parturient paresis and subsequently were treated for the disease. Two of the remaining four cows did not show symptoms of milk fever and can be considered as having normal uncomplicated parturitions. The remaining two cows did not "go down" with milk fever and were not treated, but they did appear slightly unsteady for a short period of time following parturition. The blood composition of these two cows paralleled much more closely the typical blood composition found in a milk-fever cow than that found in a normal cow at parturition. Though the latter two cows cannot be considered as having milk fever, their blood composition was so much different from that of a normal cow that to include them in normal cow averages would confuse the data. Therefore, they are treated as a separate group and are referred to hereafter as "borderline" cows.

Tables 1 and 2 present average values obtained of blood and urinary constituents of the three groups prior and subsequent to parturition.

*The blood picture at parturition.* Levels of the various blood constituents which were studied in this experiment were about the same as those previously reported in the literature for cows at parturition (2, 6, 7, 14).

The blood serum calcium dropped about 10 per cent (10 to 9 mg. per cent) in normally freshening cows and about 40 per cent (10 mg. per cent to 6 mg. per cent) in cows which developed milk fever. Blood calcium levels dropped almost as much in borderline cows as in milk-fever cows.

The blood serum magnesium in cows freshening normally did not change from prepartal levels as a result of parturition. Both the milk-fever and the borderline cows exhibited a marked increase in serum magnesium levels on the day of parturition. These values rose to still higher levels 1 day postpartum, but by 4 days postpartum the values had returned to prepartal levels.

The average drop in blood plasma inorganic phosphorus was to 4.44, 2.40 and 2.06 mg. per cent at parturition in the normal, borderline and milk-fever cows, respectively. The magnitude of the drop was not greatly different in the three groups between 2 days before parturition and day of parturition, since the normally freshening group had a considerably higher level of blood plasma phosphorus 2 days before parturition than did either of the other groups.

Levels of blood serum citric acid at calving followed the same pattern as that described by Blosser and Smith (2). There was a drop in the serum citric acid at parturition in all groups, but the milk-fever and borderline cows exhibited a greater drop than the normally-calving cows.

[4] n-Heptane used in the citric acid analyses by the method of Taussky and Shorr (25) was generously supplied by the Phillips Petroleum Co., Bartlesville, Oklahoma.

*Excretion studies at parturition—urinary calcium.* The 24-hr. urinary excretion of calcium did not seem to vary markedly in any of the three groups. No one group of the three consistently excreted excessively larger or smaller amounts of calcium via urinary channels than either of the other two groups. One of the cows, which subsequently developed milk fever, excreted in excess of 3 g. of calcium during the 24-hr. period on the tenth day prepartum. This was more than five times as much urinary calcium as was excreted by the same cow or any other cow, at any other time during the collection period, either pre- or postpartum. It is doubtful whether any significance should be attached to this variation, since it was shown by only one cow on 1 day.

There was not an inverse relation between the urinary excretion of calcium and the blood serum levels. The drop in blood serum calcium at parturition was not accompanied by an excessive excretion of this constituent via urinary channels. Thus an explanation for the dramatic drop in blood serum calcium which occurs in parturient paresis will have to be sought for elsewhere.

*Urinary magnesium.* There were distinct differences in the prepartal urinary excretion of magnesium by the milk-fever and borderline cows on one hand, and the normal cows on the other hand. There is not a sufficient amount of data to compare the three groups more than 5 days prepartum.

The cows which subsequently developed milk fever excreted larger amounts of magnesium via the urinary route between 16 and 3 days prepartum than did any of the cows of either of the other groups at any time, either pre- or postpartum. Daily excretion of magnesium was especially high in the milk-fever cows studied between 15 and 8 days prepartum. The urinary excretion of magnesium was quite constant between 7 and 3 days prepartum, following which there was a considerable drop on the second day prepartum. There was a still further decline in magnesium excretion on the first day prepartum. The average magnesium excretion was essentially the same on the day of parturition for the milk-fever, borderline and normal cows (milk fever, 1.611 g.; borderline, 1.474 g.; normal, 1.820 g.). The borderline group followed essentially the same excretory pattern for urinary magnesium as did the milk fever group, except for the fact that their greatest decline came between 1 day prepartum and the day of parturition.

The normally-freshening cows excreted magnesium at fairly constant levels prepartum, via the urinary route. Between 2 days prepartum and the day of parturition, however, there was a slight increase in urinary excretion of magnesium. This is in contradistinction to the definite decrease which occurred in both the milk-fever and borderline cows during the same time.

There was a close parallel between the normal and borderline groups for the first 2 days postpartum. Both groups showed an increased urinary excretion of magnesium during this time. However, on and following the third day postpartum, while the normal groups continued to excrete 3 g. daily of magnesium, in the borderline cows the magnesium excretion again dropped, and, on the fifth and tenth day postpartum, more closely paralleled magnesium excretion in the cows which had had milk fever than the normal cows. Thus, the magnesium

excretion in both the milk-fever and in the borderline cows was substantially less than in the normal cows on the fifth and tenth days postpartum.

If an inverse relation existed between urinary excretion and the blood levels of any of the constituents studied in this trial, magnesium was that constituent. However, the days on which the major changes occurred in each are not coincident. The greatest drop in urinary magnesium excretion in the milk fever cows occurred between the third and first days prepartum, but the greatest increase in blood serum magnesium occurred between 1 day prepartum and 1 day postpartum. Furthermore, the decline in serum magnesium levels to normal, following parturition, was not accompanied by a substantial increase in urinary excretion of magnesium. In addition, while the serum magnesium levels remained essentially unchanged in normal cows over a period of time extending from 5 days prepartum to 5 days postpartum, the urinary excretion of magnesium nearly doubled during that time.

Calculations show that changes occurring between days in urinary magnesium excretion at the time of parturition could easily account for blood changes in magnesium of the magnitude observed in milk fever. However, in view of the information presented above, and since considerable magnesium also is excreted via fecal channels and in the milk, a complete balance study would be necessary to determine whether or not urinary excretion is chiefly responsible for the blood changes in magnesium at parturition.

*Urinary citric acid.* There were pronounced differences between the milk fever group and the normal and borderline groups in the excretion of citric acid. No significance can be attached to the differences in urinary excretion of citric acid between the groups more than 5 days prepartum, since only a few comparisons are available. However, on the third, second and first days prepartum, there was consistently a greater excretion of citric acid in the cows which subsequently developed milk fever than in either the normal or the borderline cows. This is particularly interesting in view of the increased levels of blood serum citric acid which were demonstrated to occur prepartum in cows subsequently developing milk fever (2).

In the normal and borderline groups, urinary excretion of citric acid was not appreciably different during the first 4 days postpartum than for the 4 days immediately preceding parturition. Only scanty data on postpartum urinary excretion in milk-fever cows are available in this study, since urine collections were not usually made for the first 4 days postpartum if the cow developed milk fever. On the fifth and tenth days postpartum, urinary excretion was essentially the same in all groups.

A rise in the urinary excretion of citric acid was not accompanied by a fall in blood serum citric acid. For the most part there seemed to be a direct relation between urinary excretion and blood serum levels, particularly in the milk fever group. Thus, an increase in urinary excretion of citric acid was accompanied by an increase in blood serum levels in the milk-fever cows.

***Urinary inorganic phosphorus.*** Maynard (17) has pointed out that the herbivores excrete very small amounts of phosphorus through urinary channels.

Ninety-four per cent of the urinary phosphorus excreted is in inorganic form.

Very small amounts of phosphorus were excreted in the urine at any time prepartum by any of the groups. The usual amounts excreted during this time were from 0.02 to 0.04 g. daily. There was no change in the urinary excretion of phosphorus postpartum in the normal group, as compared with prepartal excretion in the same group.

There was a very dramatic increase in inorganic phosphorus excretion by both borderline cows on the second day postpartum. These two cows excreted about 5.0 g. on the average for this day. This was more than 100 times as much inorganic phosphorus as was excreted by the same cows at any other time prepartum and about ten times as much inorganic phosphorus as was excreted by the same cows at any other time postpartum.

On the fifth day postpartum, the milk fever group excreted considerably more inorganic phosphorus than at any other time pre- or postpartum, but on the tenth day postpartum, urinary excretion of this constituent had returned to its normal low levels. On the other hand, the borderline group was still excreting substantially larger amounts on the tenth day postpartum than they had excreted at any time prepartum. This amount also was considerably in excess of that excreted by either of the other groups on the same day.

No definite relationship existed throughout the study between blood plasma inorganic phosphorus and the urinary excretion of this constituent. In the borderline group, however, for each increase in the daily urinary excretion of inorganic phosphorus there was a concurrent increase in the blood plasma levels. This relationship held for every day postpartum during which blood samples were taken and urine collections were made.

It is important to note specifically that the trends observed in the preceding paragraphs are based on a relatively small number of cows. The behavior patterns of the various groups, insofar as urinary excretion is concerned, might change somewhat, particularly in excess of 5 days prepartum, if further studies were made. The fact that the blood composition, even with a small number of cows involved in the average, was quite typical of both normal and milk-fever cows and that the urinary excretion data was quite consistent for cows of the different groups, leads one to believe that the urinary excretion data at parturition as established in this study is quite typical of that which would be found if more cows were studied.

## SUMMARY

Urinary calcium excretion by eight Jersey cows was quite variable at parturition. There was no consistent or appreciable variation between the normal, milk-fever and borderline cows. Neither did a relation exist between calcium losses via urinary channels and changes in the blood calcium.

Urinary magnesium losses were much more extensive between days 16 and 3 prepartum in the cows which developed milk fever than in the normal cows. The borderline cows were intermediate between the other groups in this respect. All three groups were excreting essentially the same amounts of magnesium via the urinary route on the day of parturition. Following parturition, the mag-

nesium excretion by the normal group increased, and by days 5 and 10 postpartum, the normal cows were excreting more urinary magnesium than either the milk-fever or borderline groups. In general, blood serum magnesium seemed to rise in the milk-fever and borderline cows as the urinary excretion of this constituent decreased, but this relation was not consistent throughout the trial.

There was approximately five times as much citric acid excreted in the urine of the cows which subsequently developed milk fever on days 3, 2 and 1 prepartum, as was excreted in the urine of the normal or borderline cows on those days. The high levels of citric acid excretion were accompanied by levels of blood serum citric acid in the milk-fever cows which were higher than normal.

Very small and consistent amounts of inorganic phosphorus were excreted by all of the cows prepartum, with no group differences. In general, the postpartal excretion also was very small. There was no apparent relation between blood plasma levels of inorganic phosphorus and the urinary excretion of this constituent.

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# CHANGES IN MILK PRODUCTION WITH AGE AND MILKING FREQUENCY[1]

JAY L. LUSH AND ROBERT R. SHRODE[2]
*Iowa State College, Ames*

## INTRODUCTION

It is well known that milk production increases with age at an ever-decreasing rate until maximum production is reached at around 6 to 8 yr. Production then declines with advancing age. This makes the regression of production on age distinctly curvilinear but the nature and amount of that curvature does not appear deducible from any general physiological principles. Consequently, age-changes have been estimated empirically from experiments or from non-experimental data thought suitable. Real but minor differences between breeds in these age-changes have been indicated by some evidence. Indeed such differences are to be expected if breeds really differ in their rates of maturity.

The present investigation primarily was undertaken to measure how production changes with age, using a method thought to be nearly free from the effects of concurrent selection. At least, whatever bias is left by this method would be in the direction opposite to the bias in most of the earlier methods. Kendrick (4) appears to have used the same method, at least in part, but did not publish the details of his procedure.

The effect of variation in times milked per day was studied also because the question is important and the data which had been arranged for the primary purpose also seemed suitable for this with only a little extra work.

## THEORETICAL CONSIDERATIONS

The primary purpose of correction factors is to remove phenotypic differences which occurred because the environmental conditions were not uniformly those chosen as standard. The correction factors ought not to remove from the records any differences really caused by things inherent in the cows themselves. However, the inherent and the environmental causes of differences are often confounded, especially in non-experimental data, so that it is difficult or impossible to separate the effects of one cleanly from the effects of the other. For example, if at each age some cows with low records are culled, then the older cows will include a larger fraction of those with inherently high production and a smaller fraction of inherently low producers than are among the cows which make records at the younger ages. If the regression of production on age is computed from the averages of all data available at each age, that curve will not show the effects of age alone but will show those effects combined with whatever effects such culling actually had.

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[2] Now Associate Professor of Genetics, Texas Agricultural and Mechanical College.

An opposite bias is introduced if the average inherent productivity of the dairy population is increasing. In that case, at any given date the averages for the older ages do not yet include records from the cows born in the most recent years when the average productivity of the population had become higher. In this way the effects of any genetic time-trends in inherent productivity may be confounded with the effects of age.

As another example of confounding, consider estimating the effects of milking three times per day instead of twice a day by merely comparing the average production of all 3× and all 2× cows. In some of the herds the policy may have been to milk thrice a day those cows which start out to produce well, but to milk only twice a day those cows whose initial production seems too low to make the extra milking worth while. To the extent that such a policy is followed, the differences in production of the 2× and 3× cows will be, in part, the causes of whether the cows were milked twice or thrice, instead of the other way around.

Where cause and effect can be intertangled so intricately, it is difficult to know whether one has measured the true size of each separate effect, especially on non-experimental data collected from the field. Yet experiments designed expressly to prevent such confounding would need to be on an expensively large scale, if they are to yield accurate results. This is so because the variability between cows is large and the repeatability of a cow's production from one lactation to another is moderately low, even when every effort to repeat the environmental conditions is made.

Correction factors may be called *statistical control* which is used instead of some *physical control* which was not actually achieved. Both kinds of control are intended to remove variations caused by circumstances thought not pertinent to the question being investigated. Physical control is the more expensive—often vastly more so—and frequently is not possible. Statistical control is subject to error from the correction factors being imperfect and, of course, can be applied only to sources of variation which were known. Physical control also is far from perfect, as is well appreciated by anyone who has ever tried to hold conditions constant for a group of cows through two or more successive lactations and then has measured the correlation between their records in one lactation and in another. That correlation should be +1.0 if one really did succeed in maintaining all the pertinent conditions constant. But usually it is only of the order of +0.4 to +0.5 for fat or milk and somewhat higher for test.

When using either physical or statistical control, some knotty questions arise if there are genuine interactions between the inherent differences in the cows and the variations for which control or correction is to be made. For example, if cows differ genuinely in their rate of maturity for milk production, then age correction factors, even though accurate for the average, will over-correct for some individuals and correspondingly will under-correct for others. The difficulty cannot at all be avoided by physical control, *i.e.*, by comparing only records made at the same age. For, even if one knew that cow A would mature at a younger age than cow B, how would one then decide whether to compare them in first lactations, or as 5-yr.-olds, or at some other age? It seems impossible

to determine differences in rate of maturity for individual cows, although that can be done for genetically distinct groups, such as breeds. Another example of interaction would occur if some cows genuinely respond more to an extra milking than do others. Then the individual cows would rank differently when milked twice and when milked thrice a day. Statistical control by using an average correction would not rank them the same as physical control by milking them all the same number of times. Yet one wishing to use the physical control may have difficulty in deciding which milking frequency he should use. The soundest general criterion is to use as standard the same kind of environment he thinks will be used by the customers for whom he is breeding these cattle. But what if he thinks some of his customers will use 2× and others will use 3× milking? Fortunately for practical use, it appears that these "interactions" are triflingly small in comparison to other uncontrolled causes of variation in the records, although they do occur.

The bias which concurrent selection introduces into age correction factors derived from the averages of all records made at each age (method A) already has been mentioned. The reasons for this bias generally are well understood. Some have thought to escape it by comparing only records made by the same cows at two successive ages (method B), as we have done here. But, when method B is used, concurrent selection introduces a bias in the opposite direction. Since that is not generally so well understood, a comparison of the biases in methods A and B seems appropriate here.

Let $N$ = number of cows which have a record at a given age; $k$ = number of these which are kept to make a record at the next age; $c = N-k$ = number of cows which die or are sold or culled before they make another record; $K$ = the mean production of the $k$ cows at the first age; $K'$ = the mean production of the $k$ cows at the next age; $C$ = the mean production of the $c$ cows at the first age; and $C'$ = the mean production which the $c$ cows would have had in the next lactation if they had been kept.

Then the true age change, free of the effects of selection, would have been $\frac{kK'+cC'}{N}-\frac{kK+cC}{N}=\frac{k}{N}(K'-K)+\frac{c}{N}(C'-C)$. Under method A, the apparent age change is $K'-\frac{kK+cC}{N}$. From this it follows that the bias (true change minus apparent change under method A) is $\frac{c}{N}(C'-K')$. Under method B the apparent age change is $K'-K$. From this it follows that the bias (true change minus apparent change under method B) is $\frac{c}{N}[(C'-K')-(C-K)]$. The difference between the two methods, which is also the difference between their biases, is $\frac{c}{N}(C-K)$.

Now if all of the $c$ were culls for low production and if this culling were done wholly on the individual records of the cows and if the repeatability of individual records is $t$, then $C'-K'=t(C-K)$. In that case, the bias in method A would be

$\frac{c}{N}t(C–K)$ and the bias in method B would be $-(1–t)\ \frac{c}{N}\ (C–K)$. From this the ratio of the two biases would be $-\frac{1–t}{t}$. The minus sign merely says that the two biases are in opposite directions. If $t$ is less than 0.5, the bias in method B actually would be larger than that in method A. But culling for low production is not done wholly on individual records and many of the $c$ are not culls. Basing the culling partly on type or on the records of dam, or sisters or other relatives close enough to have any practical use for predictive value, has the same effect here as making $t$ larger and C–K smaller than they would be if the same fraction of the cows were culled wholly on their own records. The presence of non-culls in $c$ keeps the ratio $K'/K$ from being so much smaller than $C'/C$ as it would be if all of the $c$ were culled because of their own low records.

Further speculation about the relative sizes of the two biases appears fruitless until more is known about the amount and kinds of selection which actually are practiced. Seath's study (8) seems to be the only extensive one yet published in that field. That study merely determined on two groups of DHIA data the size of K–C at various ages. The size of the bias is affected by $\frac{c}{N}$ which, with an average productive life of about 4 yr. for dairy cows, would be somewhat larger than 0.25 from 1 yr. to the next. It was around 0.3 in Seath's data. The size of the bias also depends on K–C, which would be small if most of the $c$ who go do so for reasons other than their production. K–C could be fairly large at the ages, if any, when most of $c$ are culls because of their own low production. Seath found evidence that the individual culling was more intense among the 2-yr.-olds and 3-yr.-olds than at later ages. At the younger ages in his data, K–C was of the order of 55 to 70 lb. of fat or 1000 to 1600 lb. of milk. Of course, all cows must leave the herd eventually. If more culling is done at the younger ages, less room is left to cull at the older ages.

The present state of knowledge leaves uncertain the size of these biases from concurrent individual selection, but clearly they are in opposite directions in methods A and B. The true age-change therefore should be between the apparent changes yielded by methods A and B, unless still other highly important causes of change in production are closely confounded with age.

## DATA USED

The data came from the files of the Holstein-Friesian Association of America[3] in 1943 and consisted of 43,573 Herd Improvement Test records from 11,001 cows. Both 2× and 3× records were included, 20,893 being 2× records from 5,374 cows and 22,680 being 3× records from 5,627 cows. Each record, coded to the nearest 10 lb., was the total milk yield within 365 days after freshening.[4]

[3] The authors are indebted to H. W. Norton, Jr., for making these data available.

[4] Since 305-day records now are used much more widely than 365-day records and this change seems likely to be permanent, some details about the general applicability of the findings must remain a bit uncertain. Many of the records did end within 305 days, only about 40 per cent extending for a full 365 days. The average length of HIR records, as found in another study, was about 324 days.

The 2× and 3× records ranged from 3,500 lb. to 25,000 lb. and from 3,600 lb. to 34,000 lb., respectively. The respective means were 11,404 lb. and 13,876 lb. Table 1 shows the average production for the various ages grouped as is customary in the breed association procedure. The age trend in these averages is shown graphically in the upper left corner of figure 3.

TABLE 1

*Averages of all records at various ages*

| Age group | Milk yield | |
|---|---|---|
| | 2× milking | 3× milking |
| | *(lb.)* | *(lb.)* |
| Jr. 2 | 9,471 | 12,318 |
| Sr. 2 | 10,046 | 12,578 |
| Jr. 3 | 10,419 | 12,700 |
| Sr. 3 | 11,046 | 13,373 |
| Jr. 4 | 11,409 | 13,732 |
| Sr. 4 | 11,912 | 14,167 |
| Jr. 5 | 12,093 | 14,493 |
| Sr. 5 | 12,168 | 14,491 |
| 6 yr. | 12,323 | 14,803 |
| 7 yr. | 12,444 | 14,813 |
| 8 yr. | 12,248 | 14,844 |
| 9 yr. | 12,044 | 14,919 |
| 10 yr. | 11,830 | 14,532 |
| 11 yr. | 12,254 | 14,396 |
| 12 yr. | 11,947 | 14,261 |
| 13 yr. | 11,531 | 14,298 |
| 14 yr. | 12,660 | 13,918 |
| 15 yr. | 11,814 | 14,889 |

Each record was taken from the files without regard to its magnitude, the only requirement being that a cow have at least three consecutive 2× records or at least three consecutive 3× records. The year or the herd in which the cow made her record was not copied from the original data file. Hence, the herd-to-herd and general year-to-year differences could not be measured separately. Requiring that the cow must have had three consecutive records on the same milking frequency must have excluded practically all cows from herds in which both 2× and 3× milking were being done at the same time. It seems likely that individual managemental or seasonal variations in environment which might have affected the regression of production on age would have been about the same within the 2× and within the 3× data. So large a sample of herds probably was fairly representative of HIR herds which are on test 4 consecutive yr. or more.

## TREATMENT OF DATA

The change in production with increasing age was measured by the ratio between production at one age and production by the very same cows in their next lactation. The 2× and 3× data were studied separately but later were combined for deriving the age correction factors, since no certain difference was apparent except at ages under 36 mo. The two types of data provided 13,802 and 14,170 pairs of records.

The ratios were computed for every monthly age level in the data by assembling in one group all cows which had one lactation beginning at $x$ months and another lactation beginning not more than 16 mo. later. Their total production ($T_1$) in their lactations beginning at $x$ months and their total production ($T_2$) in their next lactations and the average interval ($12+m$) in months between the beginning of the first and the beginning of the next lactations were computed. Then $T_2$ was adjusted to $T$ for an interval of exactly 12 mo. by a linear interpolation thus:

$$(1) \quad T = T_1 + \frac{12}{12+m}(T_2 - T_1).$$

Then $R$, the desired ratio of production at one age to what it was 12 mo. earlier was obtained by dividing (1) by $T_1$ as is shown in equation (2). This was convenient for computing.

$$(2) \qquad R = \frac{T}{T_1} = 1 + \frac{12}{12+m}\left(\frac{T_2}{T_1} - 1\right)$$

As a numerical example, the computations were as follows for the 284 2× pairs in which the first records began when the cows were 24 mo. old:

$$R = \frac{T}{T_1} = 1 + \frac{12}{12.5}\left(\frac{296{,}469}{262{,}779} - 1\right)$$
$$= 1 + 0.96\ (1.13-1)$$
$$= 1.12$$

This $R$ shows the slope of the regression of production on age ($x$) through the relation: $R - 1 = \frac{\Delta y}{y} \doteq \frac{dy}{dx} \cdot \frac{\Delta x}{y}$ where $y$ is the yield at the age when the first record of the pair began but $\frac{dy}{dx}$ applies to the curve approximately 6 mo. later and $\Delta x$ is 12 mo. As long as $R$ exceeds 1.0, $\frac{dy}{dx}$ is positive and production is still increasing with age. At the age of maximum production $\frac{dy}{dx}$ is 0 and $R$ is 1.0. When production starts to decline, $R$ becomes less than 1.0. In these data the average value of $m$ was a little less than 1 mo.

The numbers of record pairs at the various monthly age levels ranged from one to 331 in the 2× data and from one to 353 in the 3× data. In the combined 2× and 3× data the number of pairs varied from one to 665. The first records of the pairs were made at ages ranging from 16 to 182 mo. The pairs beginning under 24 mo. or later than 104 mo. were so few that the ratios at these ages could vary widely just by chance. This was minimized by using moving averages and by combining the ratios at extreme ages, as is explained later. In one-fifth of the pairs the first ages were under 36 mo. Half of them began at ages under 50 mo. A fifth of them began at ages as old as 72 mo., but only one-twentieth of the information was for pairs beginning at ages as high as 104 mo. At the youngest age and at the ages so old that the data were scarce, enough consecutive months were combined to have at least 20 cows as the basis for each $R$ in the 2× data. The same ages were used for grouping the 3× data.

To reduce the effects of sampling fluctuations, a 5-mo. weighted moving average was fitted to both the 2× and 3× ratios. The relative weights for the 5 mo. were 1–3–4–3–1 in the 2× data, but the slightly different weighting, 1–2–3–2–1, was used later in the 3× and combined data, as it was found simpler to compute and the choice of weights had to be somewhat arbitrary anyhow. In all the weighting the ratio for each month was multiplied by the number of record pairs involved. This gave weight to the amount of evidence on which each $R$ was based. These weighted average $R$'s are shown in figure 1.

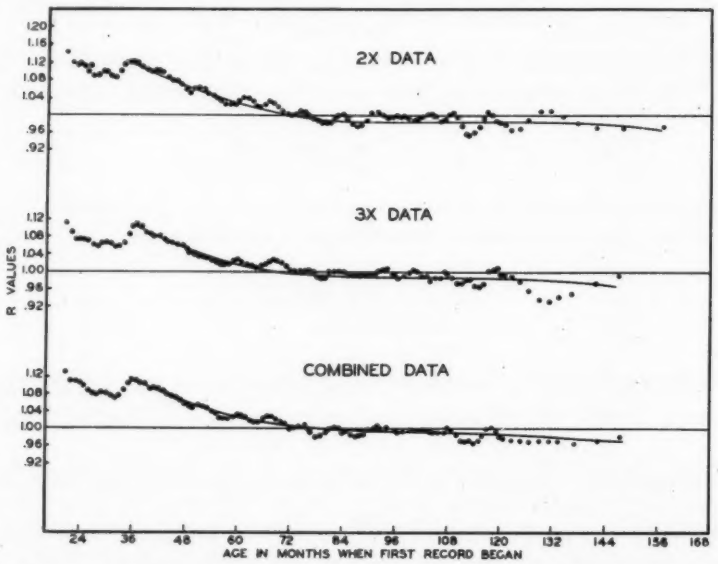


FIG. 1. Ratio of production at one age to production twelve months earlier.

The salient things about figure 1 are (a) the smoothness and simplicity of the curve for ages over 36 mo. and (b) the distinct sag between 24 and 36 mo. These things are so nearly alike in the 2× and the 3× data that they must be real and are not mere sampling accidents. That the up-and-down scatter of the points is slight, except at ages past 108 mo. where the data are few, indicates that only small sampling errors remain here.

That $R$ changes in a slightly undulating way is partly a consequence of using moving averages. Adjacent months have six of their nine elements alike where the 1–2–3–2–1 weighting was used, points with 1 intervening month have four elements alike, those with two intervening have two elements alike, and even those with 3 intervening months still have one of their nine elements in common. But points with 4 or more intervening months are entirely independent, except as a second record in one pair was sometimes an initial record in some later pair.

What the sag before 36 mo. means concerning the shape of the age curve is

shown in figure 2 in the curve for "present" factors. Its plausible biological explanation will be discussed later.

The $2\times$ and the $3\times$ data in figure 1 follow a closely similar course, although the $2\times R$'s are distinctly larger than $3\times R$'s at ages under 36 mo. The $2\times R$'s are a little larger than the $3\times R$'s at ages from 36 to about 63 mo., but this seems too uncertain and too small to warrant constructing separate sets of correction factors. The difference means that under 36 mo. the $2\times$ curve should be a bit steeper and the $3\times$ curve correspondingly not quite as steep as the curve shown in figure 2 for "present" factors. A similar but extremely small difference occurs from 36 mo. to a little after 60 mo. The reason for the difference at ages under 36 mo. is apparent in figure 4.

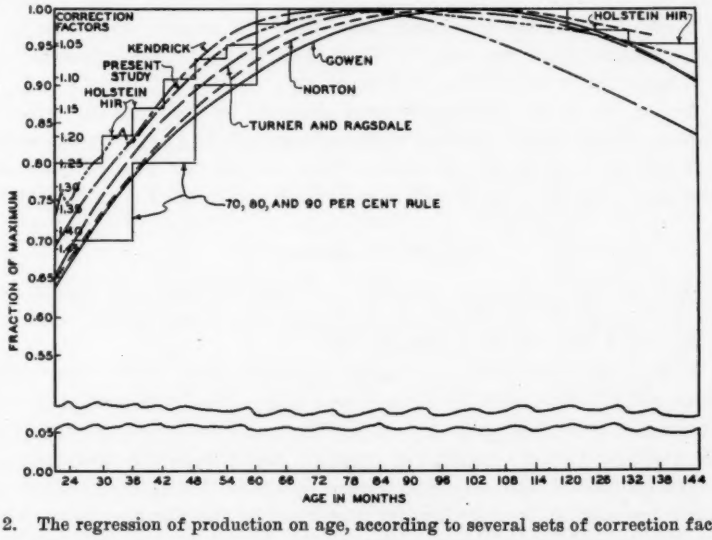


FIG. 2. The regression of production on age, according to several sets of correction factors.

The smooth curves in figure 1 for ages from 37 mo. onward were fitted by eye because of uncertainty about what type of equation to choose for fitting by precise mathematical methods and because unsatisfactory results were obtained, especially near the ends, when trying to fit polynomial curves.

The factors presented in table 2 and used in figures 2 and 3 were derived from the smooth curve fitted to the combined $2\times$ and $3\times$ data for ages of 37 mo. and over. The first step in deriving these factors was to find the point of maximum production, $M$, on the age-production curve. This would be about 6 mo. later than the age at which $R$ becomes 1.0. Figure 1 makes it clear that $M$ could not have been earlier than 78 nor later than 84 mo. This has been taken to be 82 mo., but $R$ is so nearly 1.0 for quite a distance here that mistaking the exact value of $M$ by as much as 4 mo. would have made only tiny differences in the age curve. The factor for converting to their mature equivalents the records begun at the age $M$ minus 12 mo. is $R$ at the age $M$ minus 12 mo. The factor

for converting records begun at age $M$ minus 24 mo. is found by multiplying $R_{M-12}$ by $R_{M-24}$. The factor for $M$ minus 36 mo. is given by the product, $R_{M-12} \cdot R_{M-24} \cdot R_{M-36}$. The factor for $M$ plus 12 mo. is the reciprocal of $R_M$, that for $M+24$ is the reciprocal of $R_M \cdot R_{M+12}$, etc. This step-wise procedure was used to locate points at 12-mo. intervals on each side of $M$.

Points on the age curve at $M-3$, $M-6$ and $M+3$ were then located by considering the apparent general shape of the curve on both sides of $M$ and interpolating. Then points at successive 12-mo. intervals on each side of $M-3$, $M-6$ and $M+3$ were found by using the $R$ values read from the curve in figure 1, just as was done for locating the points at 12-mo. intervals from $M$. This gave at 3-mo. intervals the points on an age curve all the way from 37 mo. to 160 mo., although the scantiness of data at the advanced ages makes a considerable uncertainty about points for ages much past 10 yr. A smooth curve was fitted by hand to these points 3 mo. apart. Thus, was obtained the "present" curve shown in figure 2 for ages 37 mo. and over.

The points for 36 mo. and under were obtained by applying the actual moving average shown in the combined data in figure 1 to the point on the smoothed curve at the age exactly 12 mo. later. This seemed the only feasible way to treat the sharp discontinuity which is so prominent in figure 1 at or just before 36 mo.

## RESULTS CONCERNING AGE

The factors thus derived are in table 2. Figure 2 shows these factors in comparison with some of the other previously published and widely used factors which have been derived from Holstein data.

As a rough and imperfect but easily understood test of how these various factors actually fit the HIR data, they were applied to the averages in table 1. The results are shown in figure 3. If (a) the factors were entirely correct and (b) the average intrinsic producing abilities really were the same for all the age-groups in table 1, the corrected curves in figure 3 should be horizontal except for minor sampling irregularities permitted by the averages being based on only moderate numbers of cows. But condition (b) is not expected to hold exactly. Individual culling probably will have made the cows in the older groups average somewhat higher in their production. This would make accurately corrected lines rise a little with increasing age. This rise might have been offset in part by a general increase in the average intrinsic productivity of the breed whereby, for example, the average productivity of all young cows born in 1935 would have been higher than the average of all those born in 1930. A few other, probably minor, circumstances might keep perfectly corrected lines from being absolutely horizontal.

## RESULTS CONCERNING FREQUENCY OF MILKING

Figure 4 shows the $2\times : 3\times$ ratios by 3-mo. intervals. Only the first records from all the pairs were used. This assured that none of the records in figure 4 would have been terminal records of the cow, such as might possibly have been affected by any udder trouble or other ill health just preceding her death or

culling. For example, the 735 2× cows who freshened at ages 23, 24 or 25 mo. and had another 2× record beginning not more than 16 mo. later, averaged 9,263 lb. of milk in their records begun at an average age of 24 mo. The 561 3× cows who freshened at ages 23, 24 or 25 mo. and had another 3× record beginning not more than 16 mo. later, averaged 11,691 lb. of milk in their first lactation. The ratio of this 9,263 to 11,691 is the 0.792 shown.

TABLE 2

*Age correction factors from the present study (not smoothed for ages under 37 mo.)*

| Age | Factor | Age | Factor | Age | Factor |
|---|---|---|---|---|---|
| (*yr.-mo.*) | | (*yr.-mo.*) | | (*yr.-mo.*) | |
| 1-9 | 1.36 | 4-3 | 1.07 | 10-4 to 10-9 | 1.04 |
| 10 | 1.34 | 4 | 1.06 | | |
| 11 | 1.34 | 5 | 1.06 | | |
| 2-0 | 1.33 | 4-6 to 4-8 | 1.05 | 10-10 to 11-2 | 1.05 |
| 1 | 1.31 | | | | |
| 2 | 1.28 | | | | |
| 2-3 | 1.26 | 4-9 to 4-11 | 1.04 | 11-3 to 11-7 | 1.06 |
| 4 | 1.25 | | | | |
| 5 | 1.24 | | | | |
| 2-6 | 1.23 | 5-0 to 5-2 | 1.03 | 11-8 to 11-11 | 1.07 |
| 7 | 1.22 | | | | |
| 8 | 1.20 | | | | |
| 2-9 | 1.20 | 5-3 to 5-6 | 1.02 | 12-0 to 12-3 | 1.08 |
| 10 | 1.21 | | | | |
| 11 | 1.21 | | | | |
| 3-0 | 1.21 | 5-7 to 6-0 | 1.01 | 12-4 to 12-6 | 1.09 |
| 1 | 1.20 | | | | |
| 2 | 1.18 | | | | |
| 3-3 | 1.17 | 6-1 to 7-8 | 1.00 | 12-7 to 12-10 | 1.10 |
| 4 | 1.16 | | | | |
| 5 | 1.15 | | | | |
| 3-6 | 1.14 | 7-9 to 8-9 | 1.01 | 12-11 to 13-0 | 1.11 |
| 7 | 1.13 | | | | |
| 8 | 1.12 | | | | |
| 3-9 | 1.11 | 8-10 to 9-7 | 1.02 | 13-1 to 13-3 | 1.12 |
| 10 | 1.11 | | | | |
| 11 | 1.10 | | | | |
| 4-0 | 1.09 | 9-8 to 10-3 | 1.03 | 13-4 | 1.13 |
| 1 | 1.08 | | | | |
| 2 | 1.08 | | | | |

The averages in figure 4 are all for 3-mo. periods except the very first one and the last five. For these six points wider time intervals were pooled in order to secure larger numbers. For example, the very first ratio shown is that between the averages of 133 cows milked twice daily and 46 cows milked three times daily, freshening at ages 17 to 22 mo. Their freshening ages averaged barely under 21 mo. At the other extreme the ratio at the oldest age is that between 31 2× cows and 27 3× cows freshening at ages from 12 yr. and 8 mo. to 15 yr. and 2 mo. and averaging 13 yr. and 6 mo.

The amount of evidence bearing on each ratio is considered as proportional to the harmonic mean of the two numbers involved and is indicated roughly on

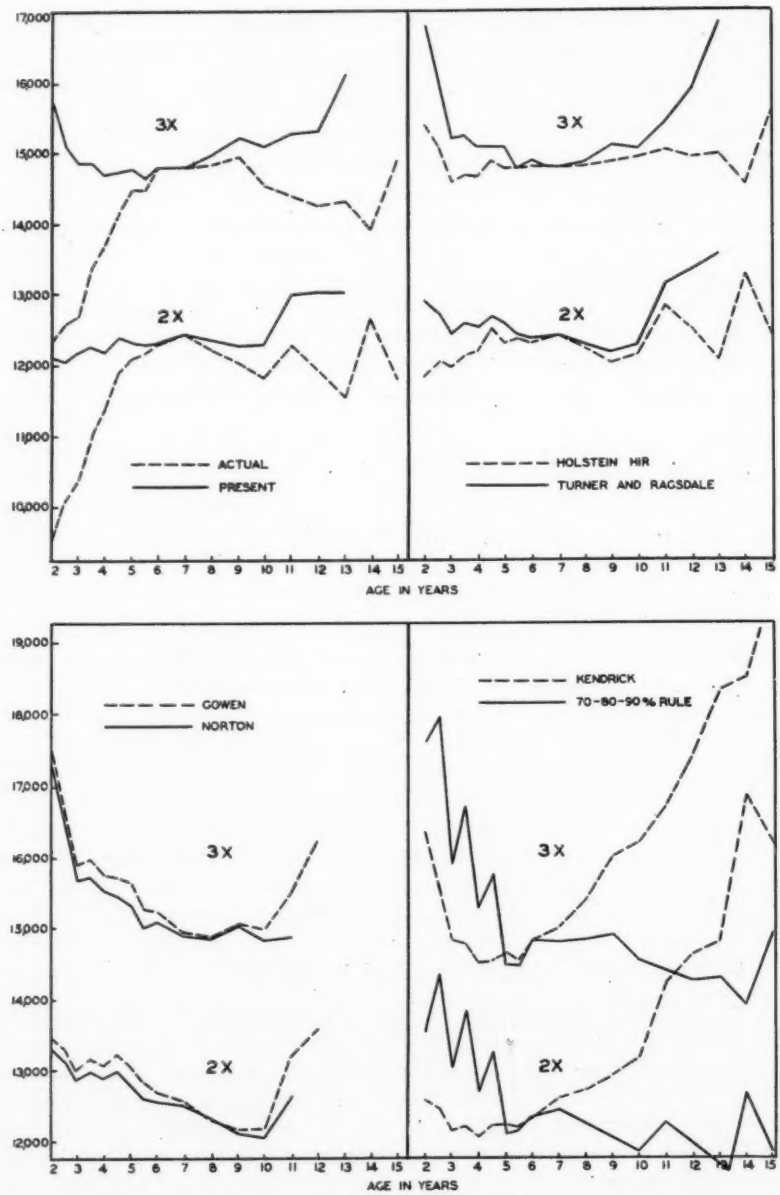


FIG. 3. The data of Table 1, actual and when corrected by the factors in Figure 2.

the graph. Only the last point is based on less evidence than the equivalent of 64 cows on each kind of milking. Two of the earlier points contain more evidence than if there were 900 cows on each milking frequency.

The horizontal line in figure 4 is at 0.833 which is the 5:6 rule so widely used for correcting 3× to 2× milking. This 0.833 seems to fit these data at 36 mo. and older ages well enough that practically nothing would be gained by changing it.

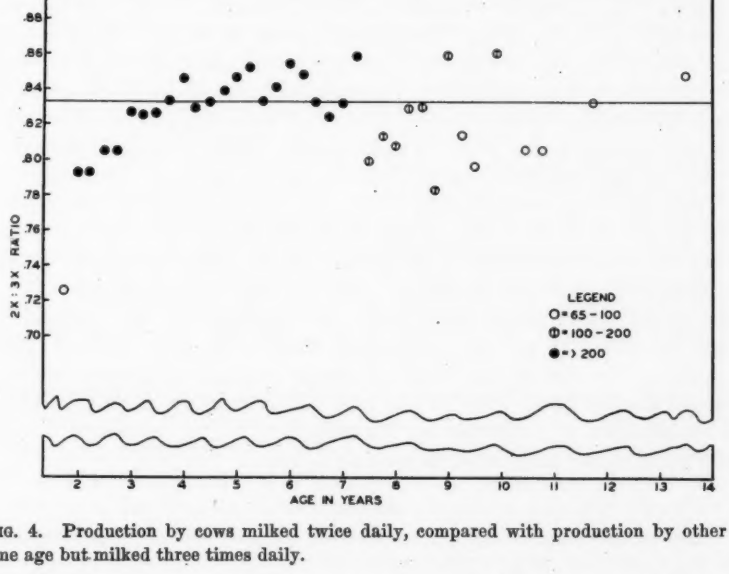


FIG. 4. Production by cows milked twice daily, compared with production by other cows the same age but milked three times daily.

At ages younger than 36 mo. the 2× averages are distinctly less than five-sixths of the 3× averages. The consistency of this and the amount of data make it certain that a different 2× : 3× correction is needed at ages under 36 mo. Using the factor 0.80 in first lactations and 0.833 in all other lactations to correct 3× records to a 2× basis would fit these data rather well[5] and would be simple enough for widespread general use. It might be imagined that the difference found in the 2× : 3× ratio at the young age is peculiar to 365-day records, many of which were in the present study, and that it does not occur in 305-day records. First lactations are known to be more persistent than later ones. But it seems more plausible that the extra milking generally would affect the first part of a heifer's record more than the latter part. This would follow if the extra milking affects yield primarily by relieving pressure in the udder. The udder capacity is relatively undeveloped at the beginning of the first lactation and would handicap production more in the first 305-days than it would in

[5] However, there is a strong hint that a perfectly accurate ratio would be progressively smaller at younger ages, especially in first records beginning at ages under 24 mo.

the 11th and 12th month. If that is an important part of the picture, 305-day lactations are likely to show an even more extreme difference in the 2× : 3× ratio than was found here between early lactations and later lactations.

## DISCUSSION

All the correction factors in figure 3 over-correct the 3× records at ages under 36 mo. more strongly than they do the 2× records. This bias appears to result from the extra milking having more effect on heifer lactations than on later ones. Therefore, in their first lactations, the 3× cows need less age correction than the 2× cows do. This situation can be met by having a single set of age correction factors suited to 2× data and then using a smaller ratio for converting 3× first lactations to a 2× basis than is used for 3× later lactations. If uncertain whether the lactation was a first or a second one, there would be small error in presuming it to be a first one if it began at an age less than 36 mo.

The "present" curve in figure 2 shows what the peak in the *R* curve (figure 1) at around 36 mo. really means. The most plausible explanation for this dip in the age curve is that cows which freshened young the first time begin to freshen for the second time in considerable numbers at around 35 mo. before they have completed their body growth or restored their nutritional reserves. Up to 34 mo. nearly all of the lactations are first calvings and the "present" curve in figure 2 shows the age change, unconfounded with any effects of prior milk production. But the second lactations begin to occur in appreciable numbers at ages of around 34 mo. By 37 mo. most of the data are for second lactations. This explanation is supported by the frequency of the combined 2× and 3× record pairs at the early ages, as shown in table 3.

TABLE 3

*Distribution of record pairs by age at which the first record of the pair began*

| Age | No. of pairs | Age | No. of pairs |
|---|---|---|---|
| (*mo.*) | | (*mo.*) | |
| 16 | 1 | 31 | 399 |
| 17 | 7 | 32 | 377 |
| 18 | 15 | 33 | 290 |
| 19 | 16 | 34 | 313 |
| 20 | 28 | 35 | 396 |
| 21 | 32 | 36 | 515 |
| 22 | 81 | 37 | 604 |
| 23 | 226 | 38 | 585 |
| 24 | 473 | 39 | 635 |
| 25 | 597 | 40 | 665 |
| 26 | 617 | 41 | 616 |
| 27 | 626 | 42 | 574 |
| 28 | 624 | 43 | 578 |
| 29 | 504 | 44 | 529 |
| 30 | 462 | 45 | 477 |

The first peak at ages of 25 to 28 mo., the valley at ages 31 to 35 mo. and the second peak at ages 37 to 41 mo. all testify to the cyclical distribution of calving ages among cows up to at least their third calving. This distribution of calving

ages also conforms to the report by Plum and Lush (7) and to some unpublished data we have seen recently from the Jersey HIR.

The evidence on the shape of the age curve up to about 34 mo. is practically all based on first lactations compared with second ones. The heifers are progressively nearer to their physical maturity when they do calve the first time. At about 34 mo. the comparisons between second and third lactations begin to occur and these all pertain to cows who freshened unusually young, both the first and the second time. Because they begin their second lactations still very immature, and in many cases somewhat depleted nutritionally, their second lactations are much smaller than their third ones, and thus they yield high $R$ values at the ages when the early second lactations are beginning. The sharp rise in the $R$ value (figure 1) at ages 34 to 37 mo. is caused by the early second calvings replacing the first calvings in the evidence for that period. Then, as the age at second calving gets much later than 37 or 38 mo., most of the cows will enter on their second lactations more nearly mature and having had more time to recuperate from their first lactations. Consequently the $R$ values fall rapidly after about 37 mo. as they begin to be based on comparing third with second lactations which were made when the cows had recovered more completely from their first lactations. In the $2\times$ data there is a hint of a similar but much smaller irregularity in the $R$ curve at about 50 to 52 mo., when comparisons between third and fourth calvings would begin to occur, but the $3\times$ data do not show this.

This explanation of the sharp break in the age curve at near 35 mo. fits extremely well the findings of Johansson and Hansson (3) (see especially the graph on their page 39), who studied the regression of butterfat yield on age within first, second, third and later lactations, separately. They found that cows calving a second time at an early age produced distinctly less than those calving for the first time at that same age.

It appears, therefore, that corrections for age will be more accurate if different age correction factors are used for first calvers and for second calvers during the ages when both are present in appreciable numbers. Probably this should be done, although the amount of work and confusion involved in changing any widely used factors is so much that the magnitude of the change needs to be verified first on other bodies of data. If a change had to be made now, the simplest way to do it seems about as follows: (a) The "present" curve in figure 2 would be given the very little bit of smoothing it needs for the youngest ages up to and including 34 mo. and would be lowered a bit at those ages to make it adapted to the $2\times$ data alone, rather than to an average of the $2\times$ and $3\times$ $R$'s as at present. (b) The factor for 35 mo. would be lowered enough to make it smooth with the existing curve for 36 mo. and later. (c) The resulting factors would be used with the qualifications (1) that if a first record began at age 35 mo. or later 0.04 would be subtracted from the factor listed for that age and (2) that if a second lactation began at ages 34 mo. or earlier, 0.05 would be added to the standard correction factor which would have been used if it were a first lactation. In effect this would make the standard corrections be for first

lactations at ages of 34 mo. and younger, but for second or later lactations beginning at 35 mo. or older. This would be fairly simple in operation, since the age range in which this amounts to using two sets of correction factors would be only from about 32 to about 40 mo. When it is not known whether the lactation was a first or a second one, the standard factor for that age would be used. This would be unfair to the few unknown second calvers at 34 mo. or less and it would be unduly generous to a few unknown first calvers at 35 mo. and older, but the net accuracy would be higher than using a single set of factors, regardless of lactation order, which is unfair to all early second calvers and unduly generous to all late first calvers. If the dividing age were set 1 mo. or even 2 mo. later than between 34 and 35 mo., fewer late first calvers would get an undeservedly high correction factor, but more second calvers would be unfairly penalized. This applies only to those for which it is not known whether the lactation was a first or a second one.

The AR factors (Gowen, Norton and Turner and Ragsdale) in figure 2 show the maximum to be at a later age and they show the production at the younger ages to be relatively lower than the HIR and DHIA factors indicate. The selective nature of AR testing is enough to explain this difference. Many of the men doing AR testing would follow a general policy of testing young cows at the first convenient and promising lactation but would think it worth while to test older cows only when those seemed likely to make an outstanding record, or at least a record much higher than any they already had. That is, in AR testing there is not only selecting of cows to be tested but also some selecting of their most promising lactations. This latter kind of selection naturally would be more extreme among older cows, since they would have started more lactations.

As far as concerns any bias from selection, the Kendrick and "present" and Holstein HIR curves show the true age curve more accurately than the AR curves do, although the former two may show the maximum at slightly too young an age and be a bit too flat before that and may decline a bit too rapidly afterward, because of the bias inherent in method B. Four of the seven sets of factors appear to over-correct at least slightly at ages past 10 yr., as judged by the evidence in figure 3. The Norton and the Holstein HIR factors have little, if any, of this fault and, of course, the 70–80–90 per cent rule makes no corrections there. This over correction at older ages is most extreme with the Kendrick factors. The practical importance of age correction factors being wrong for these ages is small because so few records are made by cows this old. If the lifetime averages of those cows are used, as they should be, errors in the correction factors at ages as old as 10 yr. will lead to only tiny errors in estimating the productivity of the cows, except in those rare cases where a cow is already old when testing begins in the herd. Many of the Kendrick data came from DHIA herds which were not on HIR test, but it seems unlikely that average management or culling practices could have been so different between DHIA and HIR herds that they would be the sole causes of the Kendrick factors showing production to decline so much at the older ages. That would require that old cows be given much worse management than young or middle-aged cows in

DHIA herds but not in HIR herds, or that culling of genuinely poor producers among the cows past middle age is far more severe and effective in DHIA herds than in HIR herds. We see no reason to suppose the former to have been true to any considerable extent, while for the latter to have been the major cause of the discrepancy seems impossible in view of the limitations which reproductive rates set on the culling possibilities among cows. One trying to cull enough to make so large a change in genuine productivity would find himself practically out of cows in 2 or 3 yr. at most! It is not apparent that any difference between 305-day and 365-day records could have been responsible for this difference at the older ages; indeed, it seems that any tendency for cows to become less persistent as they grow old would have operated in the other direction. Possibly the Kendrick data include more pairs in which the second record was really a terminal one, abnormally small because of udder trouble or ill health, but we see no reason why such pairs should be much more frequent in DHIA than in HIR data at older ages but not more frequent at younger ages.

As compared to the present and the Holstein HIR factors, the Kendrick ones appear slightly too low at ages of about 45 to 66 mo., as can be seen in figure 2. This is confirmed in figure 3 for the 3× data and, at least for the junior fours, in the 2× data. The difference is small but, because it pertains to ages at which many records are made, it deserves inquiry whenever a revision of the present Kendrick factors is considered.

It is easy to exaggerate the importance of differences in age correction factors and to waste time on further refinements of those, while neglecting things which cause much larger errors in selections and cullings. As an approximate method of measuring the actual importance of age correction factors, the variance component due to differences between age groups in the uncorrected 2× data of table 1 was estimated and then compared with the amount that remained in these data after they had been age-corrected as shown in figure 3. The age component in the uncorrected data of table 1 was about 11 per cent of the individual variance. On account of the absence of young cows with only one or two records from these data and the under-representation of cows with records at extremely old ages, we suppose that 14 to 16 per cent would be a better estimate for the general importance of age in causing variations in utterly unselected records. Also some real age differences still remain in table 1 within such broadly based groups as junior twos and threes and senior twos, so that the 11 per cent found is not quite all of it. In the unselected Johansson and Hansson data (their table 5) the component between groups was also only 11 per cent of the total. But then their data were grouped only by lactation order, so that a larger share of the age variance must have remained within their groups than within the groups in table 1 here.

The simple 70–80–90 per cent rule takes out 52 per cent of the age variance which was in table 1. That such crude factors could remove so much of the age effect indicates already that even a few refinements would go far toward completing the job. The extremely step-wise nature of the 70–80–90 per cent corrections causes the sharp zigzags at the younger ages in figure 3. These cor-

rections are clearly too generous to the 2-yr.-olds and 3-yr.olds, especially to the senior ones among them.

The Kendrick factors take out 91 per cent of the age variance. Half of the 9 per cent which is left comes from the factors being too high at ages of 11 and over, while two-thirds of it comes from their being too high at ages of 9 and over. Errors at those older ages will cause little practical damage, since such cows will already have other records except in the few cases where they are in herds which are just beginning to test. About 2 out of the 9 per cent of the original age variance left by the Kendrick corrections comes from those factors being too small in the age zone from junior threes to senior fives. This 2 per cent is certainly too small to justify the confusion and clerical work of changing factors until it has been verified on other data, yet it does have a little practical importance because so many culling and selection decisions must be based on records made in that age zone.

The Holstein HIR factors retain only about 3 per cent of the original age variance and the "present" ones retain only 1 per cent, but such figures show these factors in rather too good a light, since they are being tested back against the very data from which they were wholly (the "present" factors) or in large part (the Holstein HIR factors) derived. Nearly half of the Holstein HIR discrepancy comes from the coarse grouping for junior twos and about a fifth of it comes from the factor being too large for the senior fours.

Except for the 3×2-yr.-olds and to a much lesser extent the 3×3-yr.-olds, the "present" factors give in figure 3 a slight upward slope such as would be expected, either from the bias in the method or from selective elimination of the cows having made the older groups in table 1 genuinely more productive than the younger ones. The upward trend seems a bit more extreme than the latter cause alone seems likely to have produced. Part of the upward slope in the very youngest ages in the 2× data, as well as the downward slope in the youngest ages in the 3× data, is due to our having combined the two kinds of data to derive an average $R$ curve before we learned that the 2× : 3× ratio was smaller at the younger ages, especially at 36 mo. and less. The upward trend expected because of having used paired records is shown by the Kendrick factors only from junior fours onward. Why it does not appear earlier is not clear and it can hardly be the major cause of the Kendrick curves going so extremely high at ages after six.

The step-wise nature of the Holstein HIR factors introduces some moderately important discrepancies at ages less than 48 mo. Whenever these factors are revised the steps should be shortened, at least to 3-mo. intervals. With modern computing machinery the clerical workers in the breed office would probably find little more work in using a separate factor for each month than in using a separate factor for each 3-mo. interval. The gain in accuracy from using the monthly factors would be trifling at ages past 48 mo.

How little is actually gained by extreme refinement of correction factors can be illustrated with fat production, which is only a little lower than milk in repeatability and in coefficient of variability. With an intra-herd standard

deviation of about 80 lb. of fat and a repeatability of about 0.4 in age-corrected 305-day lactation records, the standard deviation of a cow's individual records around her true productive value is $80\sqrt{0.6}$. One quarter of this is about 16 lb. The standard error of estimating her real ability from one record is $80\sqrt{0.4} \cdot \sqrt{0.6}$, one fourth of which is about 10 lb. Little information is lost by grouping data, provided the grouping interval is as small as one-quarter of a standard deviation. Hence almost no accuracy in estimating the future production of a cow is gained by considering her individual lactation fat record in any more detail than rounding it to the nearest 10 lb. To make that much difference on an actual record of 500 lb. of fat, the age-correction factor would have to be in error by about 0.02.

Small biases from age-correction factors can become important, however, when comparing the averages of groups differing widely in average age, as may often be the case in proving a sire by comparing the production of his daughters with that of their dams. For example, if nine cows were in each age group, the statistician's rough rule would give about 5 lb. (instead of 16) as the largest interval in which such averages ought to be grouped, while if there were 100 cows in each group that interval would sink to about 1.6 lb. or a little less. For such purposes errors even as small as 0.01 in the correction factors occasionally attain some practical importance. Of course there is no argument for leaving even small errors in the correction factors unless the cost of removing them (including the confusion caused by any change in factors already widely used) costs more than the gain in accuracy is worth.

Age corrections by regression, rather than by multiplicative factors have been proposed sometimes (10). These really attempt in a single operation to correct *both* for age and for imperfect repeatability, rather than for age alone as the multiplicative factors do. Gowen (2, table 23) showed long ago that the standard deviation of actual records varies from age to age nearly as the mean does. Therefore, a multiplicative correction comes nearer than a regression equation to making the corrected records equally variable at all ages. Without that equality, records corrected to maturity cannot fairly be pooled with each other or with actual records made at maturity. Allowance for imperfect repeatability does need to be made in most practical situations but usually it is simpler and more accurate to correct for age in one operation and then for imperfect repeatability in another.

The present analysis sheds no light on the argument concerning correcting for weight instead of correcting for age (1), since weights were not available. In any event, weight and age are confounded in most data so that correcting on either basis does part (although not all) of what would be done by correcting on the other.

Only the milk records were studied here, whereas some of the other studies were of fat. But milk quantity changes so much more than test does with age and with times milked that separate sets of correction factors for milk and for total fat do not seem worth while. During the latter part of the lactation the test increases slightly. This might possibly have effect enough to make some de-

tectable difference between 365-day milk factors and 305-day fat factors, especially in first lactations. Test decreases very slightly with age but this does not seem enough to have accounted for any detectable part of the difference discussed.

## SUMMARY

1. Multiplicative age-correction factors for Holsteins were derived from 43,573 Herd Improvement Registry lactations of 5,374 cows milked twice daily and 5,627 cows milked thrice daily. These factors were based on comparisons of production by the same cows in consecutive lactations. The factors are shown in table 2.

2. The biases which culling of cows may cause in age correction factors are discussed. Several sets of factors for Holsteins are compared in figure 2. Figure 3 shows the actual HIR averages before and after correction of these factors.

3. The present findings agree well with the widely used Kendrick factors. The differences indicate that the following four points need attention whenever a revision of the Kendrick factors is made:

(a) The Kendrick factors seem a bit too large at ages under 35 mo. Part of this might come merely from the higher persistency of heifers, since the present data included many lactations which went as long as 365 days. Part of it may have come from neglecting the difference between late first calvers and early second calvers in the smoothing when the Kendrick factors were prepared.

(b) Some difference needs to be made between late first and early second calvers at those ages when records by both are present; *i.e.*, at around 33 to 42 mo. This might be done rather simply by having a sharp discontinuity in the standard age-correction factors between 34 and 35 mo. and adding about 0.05 to the standard factor if the calving is a second one which occurred earlier than 35 mo., but subtracting about 0.04 from the standard factor if it is a first calving which occurred at ages later than 34 mo.

(c) The Kendrick factors appear a bit too low at ages around 45 to 66 mo. The apparent difference is small but does have a little importance, because so many of the records on which cows are culled or are chosen to be the dams of herd sires are made at these ages.

(d) The Kendrick factors seem decidedly too large at the older ages.

4. Because all of the commonly used age corrections remove most of the variance caused by age, their accuracy can be increased only a little by further changes. Since any changes involve much confusion and clerical labor, those which the present study indicates to be desirable should first be verified on other data, especially with respect to these points:

(a) Is any material portion of them the peculiar result of the present data having included many lactations as long as 365 days?

(b) Is the slope of the age curve really different enough in HIR and in DHIA data to need attention?

(c) Is the explanation proposed here for the peculiar shape of the $R$ curve at around 36 mo. really valid and is there any simpler and equally accurate device for adapting age corrections to it?

5. Multiplying 3× records by 0.80 when they are first lactations and by 0.833 when they are later lactations is suggested for correcting them to the basis of twice-a-day milking. The data hint that the difference between 2× and 3× milking is even larger than 4:5 at extremely young ages.

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(9) TURNER, C. W., AND RAGSDALE, A. C. A Comparison of Holstein-Friesian Sires. Mo. Agr. Expt. Sta. Bull. 217. 1924.

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# PROGRAM

## FORTY-FIFTH ANNUAL MEETING

OF THE

## AMERICAN DAIRY SCIENCE ASSOCIATION

CORNELL UNIVERSITY

ITHACA, NEW YORK

JUNE 20–22, 1950

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GENERAL PROGRAM COMMITTEE

W. E. PETERSEN, Minnesota, *Chairman*
C. W. REAVES, Florida
D. V. JOSEPHSON, Pennsylvania
G. M. CAIRNS, Maryland
J. M. SHERMAN, New York
K. L. TURK, New York

## GENERAL PROGRAM

*Monday, June 19, 1950*

12:00 Noon* **Open for Registration,** *Memorial Room, Willard Straight Hall*

7:00–12:00 p.m. **Registration and Informal Gathering,** *Memorial Room, Willard Straight Hall*

*Tuesday, June 20, 1950*

8:00 a.m. REGISTRATION

10:00–12:00 a.m. OPENING SESSIONS, *Bailey Hall*

K. L. TURK, *Department of Animal Husbandry, Cornell University,* presiding

**Address of Welcome**

DR. W. I. MYERS, *Dean, College of Agriculture, Cornell University*

**Presidential Address**

DR. G. M. TROUT, *Dairy Division, Michigan State College*

**Guest Speaker**

DR. E. E. DAY, *Former President and Chancellor, Cornell University*

1:30–4:30 p.m. SECTIONAL MEETINGS

**Production Section A**

Genetics

*Room 100, Caldwell Hall*

* All events scheduled are Eastern Daylight Saving Time.

**Production Section B**
Calf Nutrition
*Room 25, Warren Hall*

**Manufacturing Section**
Chemistry
*Room 233, Plant Science Building*

**Extension Section**
Opening Business Session, Teaching Methods and Exhibits, *Room 125, Warren Hall*
Extension Exhibits, *Room 101, Warren Hall*

8:00–12:00 p.m. RECEPTION AND DANCE, *Statler Hall*

*Wednesday, June 21, 1950*

9:00–12:00 a.m. SECTIONAL MEETINGS

**Production Section A**
Endocrines and Mammary Secretions
*Room 100, Caldwell Hall*

**Production Section B**
Nutrition and Physiology
*Room 25, Warren Hall*

**Manufacturing Section**
Cheese, Whey
*Room 233, Plant Science Building*

**Extension Section**
Dairy Herd Improvement Associations and Dairy Cattle Breeding
*Room 125, Warren Hall*

1:30– 4:00 p.m. SECTIONAL MEETINGS

**Production and Extension Sections** (Joint Session)
Symposium: Grassland Utilization and Its Relation to Dairying
*Bailey Hall*

**Manufacturing Section**
Microbiology, Sanitation
*Room 233, Plant Science Building*

4:00– 5:00 p.m. COMMITTEE AND BUSINESS MEETINGS

**Production Section,** *Room 25, Warren Hall*
**Manufacturing Section,** *Room 233, Plant Science Building*

8:00–10:00 p.m. ENTERTAINMENT, *Bailey Hall*

*Thursday, June 22, 1950*

9:00–11:00 a.m. SECTIONAL MEETINGS AND BUSINESS MEETINGS

**Production Section A**
Feeding
*Room 100, Caldwell Hall*

**Production Section B**
Reproduction
*Room 25, Warren Hall*

**Manufacturing Section**
Milk, Ice Cream, Concentrated and Dry Milks
*Room 233, Plant Science Building*

**Extension Section**
4-H Club Work, Committee Reports, Business Meeting
*Room 125, Warren Hall*

11:00–12:00 a.m. **Production Section Business Meeting,** *Room 25, Warren Hall*

1:30– 3:00 p.m. SECTIONAL MEETINGS

**Production Section A**
Management
*Room 100, Caldwell Hall*

**Production Section B**
Semen Techniques
*Room 25, Warren Hall*

**Manufacturing Section**
High Temperature Processing
*Room 233, Plant Science Building*

**Extension Section**
Dairy Cattle Health
*Room 125, Warren Hall*

3:00– 5:00 p.m. BUSINESS MEETING OF THE ASSOCIATION
*Room 25, Warren Hall*

7:00 p.m. ANNUAL BANQUET, INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS, *Statler Hall*
J. M. SHERMAN, *Department of Dairy Industry, Cornell University,* Toastmaster

PROGRAM OF ENTERTAINMENT

(Principally for the Ladies)

*Tuesday, June 20, 1950*

3:00 p.m. TEA, *Martha Van Rensselaer Hall*

8:00–12:00 p.m. RECEPTION AND DANCE, *Statler Hall*
(Open to all registered.)

*Wednesday, June 21, 1950*

1:00 p.m. COMPLIMENTARY LUNCHEON AND BRIDGE, *Statler Hall*

8:00 p.m. CONCERT. Endicott Johnson Workers' Chorus, *Bailey Hall* Courtesy of Endicott Johnson Corp., Johnson City, N. Y. (Open to all registered.)

*Thursday, June 22, 1950*

10:00 a.m. TOUR of Taughannock Falls and Robert E. Treman State Parks. Complimentary picnic luncheon for ladies and children

7:00 p.m. ANNUAL BANQUET, INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS, *Statler Hall.* (Open to all registered.)

## PROGRAM OF MANUFACTURING SECTION

*Tuesday, June 20*

Afternoon Session. *Room 233, Plant Science Building*

1:30– 4:30 **CHEMISTRY.** D. V. JOSEPHSON, *Chairman*

M1 Ion Exchange as a Means of Varying the Salt Constituents of Milk. H. S. HALLER AND A. G. MORIN, *Bureau of Dairy Industry, U.S.D.A.*

M2 Isolation of the Non-casein Proteins of Milk. A. R. KEMP, B. C. JOHNSON AND A. M. SWANSON, *University of Wisconsin*

M3 A Detailed Study of the Non-protein Nitrogen Fractions in Milk. K. M. SHAHANI AND H. H. SOMMER, *University of Wisconsin*

M4 A Colorimetric Determination of Lipase. (A Preliminary Report.) GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

M5 Nephelometric Determination of Fat in Non-fat Dry Milk. BURDET HEINEMANN, E. J. BALDI AND O. B. PARKER, *Producers Creamery Co., Springfield, Mo.*

M6 The Action of Mineral-ion Exchange Resins on Certain Milk Constituents. CHARLES W. GEHRKE, *University of Missouri,* and EMORY F. ALMY, *Ohio State University*

M7 Preliminary Observations on the Electrophoresis Study of the Proteins in Skimmilk. W. L. SLATTER AND Q. VANWINKLE, *Ohio State University*

M8 State of Solution of the Naturally Occurring Salts in Milk. INDRAPAL S. VERMA AND H. H. SOMMER, *University of Wisconsin*

M9 Studies on Oxidized Milk Fat. MARK KEENEY AND F. J. DOAN, *Pennsylvania State College*

M10 The Enzymatic Hydrolysis of Lactose in Dairy Products and Its Determination. FRANK E. POTTER, *Agricultural Research Administration, U.S.D.A.*

M11 Studies on the Water-insoluble Acids of Butter. F. J. BABEL, *Purdue University*

M12 The Lactometer as an Instrument for Determining Added Water in Milk. OLE YSTGAARD AND E. W. BIRD, *Iowa State College*

*Wednesday, June 21*

Morning Session. *Room 233, Plant Science Building*

9:00–12:00 **CHEESE, WHEY.** E. L. JACK, *Chairman*

M13 Rapid Salting of Brick Cheese. H. J. BUYENS AND W. V. PRICE, *University of Wisconsin*

M14 Pasteurization of Milk for Italian Cheese Curd. J. C. MARQUARDT, *New York State Department of Agriculture and Markets*

M15 The Influence of Ultrasonic Sound Waves on Cheese Ripening. W. C. WINDER, A. M. SWANSON AND W. V. PRICE, *University of Wisconsin*

M16 Observations on an Exudate from Cheddar Cheese. V. L. ZEHREN AND A. M. SWANSON, *University of Wisconsin*

M17 The Forced-drying of Cheddar Cheese Prior to Paraffining. D. M. IRVINE AND W. V. PRICE, *University of Wisconsin*

M18 The Free Amino Acids of Foreign Type Cheese. F. V. KOSIKOWSKY AND A. C. DAHLBERG, *Cornell University*

M19 The Methyl Ketones of Blue Cheese. STUART PATTON, *Pennsylvania State College*

M20 Tyramine Production in Cheese and in Various Bacterial Cultures. JOHN A. HUPFER, JR., GEORGE P. SANDERS AND RALPH P. TITTSLER, *Bureau of Dairy Industry, U.S.D.A.*

M21 The Effect of Penicillin and Streptomycin on Swiss Cheese Starters. R. E. HARGROVE, H. E. WALTER, J. P. MALKAMES AND K. T. MASKELL, *Bureau of Dairy Industry, U.S.D.A.*

M22 Observations on a Gelatinous Curd Type of Spoilage of Cottage Cheese. R. B. PARKER, V. N. SMITH AND P. R. ELLIKER, *Oregon State College*

M23 Low Cost and Small Scale Methods for Concentrating Whey for Feed. A. H. STEVENS, *Bureau of Dairy Industry, U.S.D.A.*

*Wednesday, June 21*

Afternoon Session. *Room 233, Plant Science Building*

1:30– 4:00 **MICROBIOLOGY, SANITATION.** J. H. HETRICK, *Chairman*

M24 The Utilization of Whey in the Microbiological Synthesis of Riboflavin. ABRAHAM LEVITON AND EARLE O. WHITTIER, *Bureau of Dairy Industry, U.S.D.A.*

M25 Strains of *Streptococcus faecalis* Present in a Starter Used in the Manufacture of Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, *Cornell University*

M26 Bacterial Studies of the High-temperature Short-time Pasteurization of Ice Cream Mix. F. W. BARBER AND H. P. HODES, *National Dairy Research Laboratories, Inc.*

M27 The Influence of pH on Proliferation of the Lactic Streptococcus Bacteriophage. W. W. OVERCAST, F. E. NELSON AND C. E. PARMELEE, *Iowa State College*

M28 Changes in Bacteriophage and Sensitive Organism Populations in a Commercial Mixed Culture. C. E. PARMELEE, F. E. NELSON AND W. W. OVERCAST, *Iowa State College*

M29 A Study on the Psychrophilic Bacteria in Market Milk. F. A. ROGICK AND L. H. BURGWALD, *Ohio State University*

M30 Effects of Storage on Penicillin in Dairy Products. W. A. KRIENKE AND E. L. FOUTS, *University of Florida*

M31 Organic Chelating Agents as an Aid to Dairy Detergency. G. A. CLAYBAUGH AND J. M. JENSEN, *Michigan State College*

M32 A Comparison of Phosphatase Tests Using Different Buffers, Precipitants and Periods of Incubation. GEORGE P. SANDERS AND JOHN A. HUPFER, JR., *Bureau of Dairy Industry, U.S.D.A.*

M33 Phosphatase Measurements on High Temperature Vacuum Pasteurized Churning Cream and Ice Cream Mix. G. H. WILSTER AND JUNIUS COVINGTON, *Oregon State College*

M34 Factors Affecting Production of Proteolytic and Coagulating Enzyme by *Streptococcus liquefaciens.* A. T. DUDANI AND F. E. NELSON, *Iowa State College*

4:00– 5:00 **BUSINESS MEETING**

*Thursday, June 22*

Morning Session. *Room 233, Plant Science Building*

9:00–12:00 **MILK, ICE CREAM, CONCENTRATED AND DRY MILKS.** E. L. JACK, *Chairman*

M35 Taking Representative Milk Samples from Weigh Tanks. J. C. MARQUARDT, *New York State Department of Agriculture and Markets*

M36 Determination of Quaternary Ammonium Compounds in Milk and in Detergent Sanitizer and Buffered Quaternary Solutions. D. D. MILLER AND P. R. ELLIKER, *Oregon State College*

M37 Ion Exchange as a Means of Improving the Keeping Quality of Frozen Homogenized Milk. H. S. HALLER AND R. W. BELL, *Bureau of Dairy Industry, U.S.D.A.*

M38 Observations on the Effect of Additions of Heat Thickened Protein to Fluid Milk on the Creaming Phenomenon. A. C. SMITH AND F. J. DOAN, *The Pennsylvania State College*

M39 The Effectiveness of Some Antifoaming Agents in the Condensing of Skimmed Milk and Whey. J. ROBERT BRUNNER, *Michigan State College*

M40 The Antioxidant Properties of Nordihydroguaiaretic Acid in Cream Pasteurized at Various Temperatures. B. T. KARNANI, DIONISIOS A. THEOKAS AND VLADIMIR N. KRUKOVSKY, *Cornell University*

M41 Stability of Evaporated Milk as Influenced by Various Conditions of Homogenization. R. B. MAXCY AND H. H. SOMMER, *University of Wisconsin*

M42 Separation of Fat and Protein in Sterilized Milks During Storage. B. H. WEBB, E. F. DEYSHER, C. F. HUFNAGEL AND F. E. POTTER, *Bureau of Dairy Industry, U.S.D.A.*

M43 The Use of Concentrated Essence for Improving the Flavor of Strawberry and Peach Ice Cream. C. C. FLORA, L. L. DAVIS AND C. W. HOLDAWAY, *Virginia Agricultural Experiment Station*

M44 Some Relationships of the Oxidation-reduction Systems of Milk to the Keeping Quality of the Dry Product. H. A. HARLAND, S. T. COULTER AND R. JENNESS, *University of Minnesota*

M45 A Disc Method of Filtration for Roller Process Nonfat Dry Milk Solids. D. R. STROBEL AND C. J. BABCOCK, *Research Division, Dairy Branch, U.S.D.A.*

M46 Comparison of the Yield of Non-fat Dry Milk Solids when Using Three Types of Spray Drying Equipment. BEN M. ZAKARIASEN AND GUNNER E. NELSON, *Land O'Lakes Creameries, Inc.*

*Thursday, June 22*

Afternoon Session. *Room 233, Plant Science Building*

1:30– 3:00 **HIGH TEMPERATURE PROCESSING.** D. V. JOSEPHSON, *Chairman*

M47 Effect of Heating on the Diffusion of Calcium, Magnesium, Phosphorus and Citric Acid. T. D. HARMAN AND W. L. SLATTER, *Ohio State University*

M48 The Operation of a Spray Drier at High Temperature and under Pressure. V. H. TOWNLEY AND S. T. COULTER, *University of Minnesota*

M49 The Changes Produced in the Ultrafilterable Calcium, Phosphorus and Nitrogen Components of Skim Milk during Processing in a Mallory Heat Exchanger. E. A. BERNARDONI AND S. L. TUCKEY, *University of Illinois*

M50 Effect of High-temperature Short-time Heat Treatments of Milk on the Denaturation of Albumin and Globulin. J. H. HETRICK, *Dean Milk Co.*, and P. H. TRACY, *University of Illinois*

M51 Lactose Degradation in Heated Milk. STUART PATTON, *Pennsylvania State College*

3:00– 5:00 **BUSINESS MEETING OF THE ASSOCIATION**

## PROGRAM OF PRODUCTION SECTION

*Tuesday, June 20*

Afternoon Session

1:30– 4:30 Section A. **GENETICS.** G. M. CAIRNS, *Chairman*

*Room 100, Caldwell Hall*

P1 A Study of the Inheritance of Persistency in Milk Production. M. H. ALEXANDER, *University of Illinois*

P2 Some of the Effects of Calving Interval on Milk and Butterfat Production of Ayrshire Cattle. W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University*

P3 A Study of the Type Ratings of Daughters of Sires and Dams that have been Classified for Type. W. J. TYLER, *West Virginia University*

P4 Preliminary Report on the Production Records of Crossbred Dairy Cattle. J. P. LAMASTER, G. W.

BRANDT AND C. C. BRANNON, *South Carolina Agricultural Experiment Station, and* M. H. FOHRMAN, *Bureau of Dairy Industry, U.S.D.A.*

P5 Inheritance of Butterfat Test in the Beltsville Holstein Herd. JOSEPH B. PARKER AND CHARLES A. MATTHEWS, *Bureau of Dairy Industry, U.S.D.A.*

P6 A Selection Index for Fat Production Utilizing the Fat Yields of the Cow and Her Relatives. J. E. LEGATES AND JAY L. LUSH, *Iowa State College*

P7 An Index for the Effects of Certain Environmental Influences on Dairy Cattle Production. N. D. BAYLEY AND E. E. HEISER, *University of Wisconsin*

P8 Heritability of Fertility in Dairy Cattle. R. S. DUNBAR, JR. AND C. R. HENDERSON, *Cornell University*

P9 Predicting the Breeding Efficiency of Dairy Cows. DURWARD OLDS AND D. M. SEATH, *Kentucky Agricultural Experiment Station*

P10 The Frequency Distribution of Cellular Antigens in Five Breeds of Dairy Cattle. L. C. FERGUSON, E. J. LAZEAR AND FORDYCE ELY, *Ohio State University*

P11 Length of Gestation in the Five Major Breeds of Dairy Cattle. M. H. ALEXANDER, *University of Illinois*

1:30– 4:30 Section B. **CALF NUTRITION.** L. O. GILMORE, *Chairman*

*Room 25, Warren Hall*

P12 The Preparation of a Riboflavin-deficient Milk for Experimental Calf Feeding. E. G. MOODY, S. M. HAUGE AND N. S. LUNDQUIST, *Purdue University*

P13 The Influence of Rumen Inoculations on the Digestibility of Dry Matter, Cellulose and Protein in Young Dairy Calves. H. R. CONRAD, J. W. HIBBS, W. D. POUNDEN AND T. S. SUTTON, *Ohio Agricultural Experiment Station*

P14 Variations in Digestibility of Certain Characteristic Rumen Microorganisms and Some Effects of their Absence on Calves. W. D. POUNDEN AND J. W. HIBBS, *Ohio Agricultural Experiment Station*

P15 Some Chemical and Physical Characteristics of the Contents of the Alimentary Tract of Calves at Time of Birth. D. B. PARRISH AND F. C. FOUNTAINE, *Kansas Agricultural Experiment Station*

P16 Postpartum Development of Bovine Stomach Compartments and Observations on Some Characteristics of their Contents. SIDNEY P. MARSHALL, P. T. DIX ARNOLD AND R. B. BECKER, *University of Florida*

P17 The Growth of Dairy Calves on Purified Diets. G. P. LOFGREEN, *University of California*

P18 The Nutritive Value of Starch and the Effect of Lactose on the Nutritive Values of Starch and Corn Syrup in Synthetic Milks for Young Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN AND H. D. WEBSTER, *Michigan Agricultural Experiment Station*

P19 Is A. P. F. of Value in a Calf Starter for Calves Weaned from Milk at an Early Age? L. L. RUSOFF AND M. O. HAQ, *Louisiana State University*

P20 A. P. F. Supplements in Milk Replacements for Dairy Calves. J. B. WILLIAMS AND C. B. KNODT, *Pennsylvania Agricultural Experiment Station*

P21 Serum Protein Fractions of Calf Blood as Influenced by Colostrum and Skim Milk. R. F. ELLIOTT AND CECIL CONLEY, *Kentucky Agricultural Experiment Station*

P22 The Effect of Kind of Carrier and Method of Dispersion on the Absorption of Carotene by Young Dairy Calves. J. W. CROWLEY AND N. N. ALLEN, *University of Wisconsin*

P23 The Riboflavin Requirement of the Very Young Calf. GERMAIN J. BRISSON AND T. S. SUTTON, *Ohio State University*

P24 Synthesis of Certain B-vitamins in the Digestive Tract of Dairy Calves. E. M. KESLER AND C. B. KNODT, *Pennsylvania State College*

*Wednesday, June 21*

Morning Session

9:00–12:00 Section A. **ENDOCRINES AND MAMMARY SECRETIONS.** G. M. CAIRNS, *Chairman*

*Room 100, Caldwell Hall*

P25 The Effects of Estrogen and Progesterone on the Arterial System of the Uterus of the Cow. WILLIAM HANSEL, *Cornell University*

P26 The Fertility of Heifers Following Administration of Progesterone to Alter the Estrual Cycle. E. L. WILLETT, *American Foundation for the Study of Genetics*

P27 A Preliminary Report on the Role of Progesterone in the Maintenance of Pregnancy in the Cow. JAMES I. RAESIDE AND C. W. TURNER, *Missouri Agricultural Experiment Station*

P28 Relative Reactions of European and Indian Cattle to Changes in Environmental Temperature. S. BRODY, H. H. KIBLER, A. C. RAGSDALE, *Missouri Agricultural Experiment Station;* H. J. THOMPSON, *U.S.D.A.*

P29 A Study of the Effect of Two and Three Times a Day Milking Upon Milk Yield. J. G. CASH AND W. W. YAPP, *University of Illinois*

P30 Observations of the Rate of Milk Removal. K. E. HARSHBARGER, *University of Illinois*

P31 The Utilization of Acetic Acid by the Perfused Mammary Gland. G. L. MCCLYMONT AND J. C. SHAW, *University of Maryland*

P32 Variations in Residual Milk Obtainable by Oxytocin Injections. ERIC W. SWANSON AND S. A. HINTON, *University of Tennessee*

P33 Evaluation of Mammary Gland Development of Heifer Calves. J. H. BOOK, W. W. SWETT AND C. A. MATTHEWS, *Bureau of Dairy Industry, U.S.D.A.*

P34 Effects of Udder Innunction with Diethylstilbestrol on Mammary Congestion in First-calf Heifers. JOSEPH MEITES, R. E. HORWOOD, E. P. REINEKE, C. S. BRYAN AND E. S. SMILEY, *Michigan Agricultural Experiment Station*

P35 Estimation of the Thyroxine Secretion Rate without Sacrifice of the Animals. G. W. PIPES, C. R. BLINCOE AND KUANG-MEI HSIEH, *Missouri Agricultural Experiment Station*

P36 Persistence of Different Causative Organisms in Mastitis Infections. LLOYD A. BURKEY, CECELIA R. BUCKNER AND W. W. SWETT, *Bureau of Dairy Industry, U.S.D.A.*

P37 Factors Involved in the Whiteside Reaction. W. E. PETERSEN, J. F. GRIMMELL AND I. A. SCHIPPER, *University of Minnesota*

9:00–12:00 Section B. **NUTRITION AND PHYSIOLOGY.** L. O. GILMORE, *Chairman*

*Room 25, Warren Hall*

P38 The Influence of Soybean Hay on Reproduction in the Rabbit. K. A. KENDALL AND G. W. SALISBURY, *University of Illinois*

P39 A Duodenal Fistula for Physiological Studies in the Bovine. G. M. WARD, F. W. YOUNG AND C. F. HUFFMAN, *Michigan State College*

P40 Tests with Sulphur Dioxide for Forage Preservation. J. B. SHEPHERD, H. G. WISEMAN, R. E. ELY, C. G.

MELIN, C. H. GORDON; L. G. SCHOENLEBER AND L. E. CAMPBELL; W. H. HOSTERMAN, *Bureau of Dairy Industry, Bureau of Plant Industry, Soils and Agriculture Engineering, and Production and Marketing Administration, U.S.D.A.*

P41 A Comparison of Fecal Nitrogen Excretion Rate, Chromium Oxide and "Chromogen (s)" Methods for Evaluating Forages and Roughages. P. G. WOOLFOLK, C. R. RICHARDS, R. W. KAUFMANN, C. M. MARTIN AND J. T. REID, *Cornell University*

P42 A Study of the Use of Chromium Oxide and Lignin as Indicators of Digestibility. E. A. KANE, W. C. JACOBSON AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P43 The Effect of Dosage Level and Method of Administration of DDT on the Concentration of DDT in Milk. R. E. ELY AND L. A. MOORE, R. H. CARTER, H. D. MANN AND F. W. POOS, *Bureau of Dairy Industry, Bureau of Entomology and Plant Quarantine, U.S.D.A.*

P44 Studies on Casein Utilization by Young Calves by Use of Radioactive Tracers. G. P. LOFGREEN, *University of California*

P45 The Biological Activity of Alpha and Beta Caseinthyroprotein. M. B. BEHRENS, G. W. PIPES AND C. W. TURNER, *Missouri Agricultural Experiment Station*

P46 Thyroxine Content of Synthetic Thyroprotein as Determined by a Radioactive Isotope Dilution Technic. E. P. REINEKE, D. P. WALLACH AND L. P. WOLTERINK, *Michigan State College*

P47 The Influence of Thyroprotein Feeding on Gains in Body Weight of Dairy Calves. RALPH P. REECE, *New Jersey Agricultural Experiment Station*

P48 The Use of X-Rays for the Detection of Rickets in Calves. J. W. THOMAS, *Bureau of Dairy Industry, U.S.D.A.*

P49 An Approach to the Problem of the Etiology of Ketosis in Dairy Cows. B. C. HATZIOLOS AND J. C. SHAW, *University of Maryland*

*Wednesday, June 21*

Afternoon Session. *Bailey Hall*

1:30– 4:00 **JOINT MEETING OF PRODUCTION AND EXTENSION SECTIONS**

G. M. CAIRNS AND C. W. REAVES, *Co-chairman*

Symposium—Grassland Utilization and its Relation to Dairying

1. The Significance of Grassland Farming in the Dairy Economy. F. B. MORRISON, *Cornell University*
2. The Composition and Conservation of Forages. L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*
3. Pastures and the Dairyman. V. G. SPRAGUE, *Northeastern Regional Pasture Laboratory*
4. The Extension Dairyman and Grassland Farming. GEORGE WERNER, *University of Wisconsin*

4:00– 5:00 **JOINT COMMITTEE REPORTS**

The Program of the Purebred Dairy Cattle Association. FRED IDTSE, *Secretary, Purebred Dairy Cattle Association*

Breeds Relations. A. R. PORTER, *Chairman*

Dairy Cattle Health. JOE NAGEOTTE, *Chairman*

Dairy Cattle Breeding. J. S. TAYLOR, *Chairman*

Type Classification. L. O. GILMORE, *Chairman*

*Thursday, June 22*

Morning Session

9:00–11:00 Section A. **FEEDING.** G. M. CAIRNS, *Chairman*
*Room 100, Caldwell Hall*

P50 Kentucky 31 Fescue as a Dairy Pasture in Northern Ohio. AVERY D. PRATT AND JAMES L. HAYNES, *Ohio Agricultural Experiment Station*

P51 Fourteen Years with Supplementary Pastures. N. R. THOMPSON AND C. W. HOLDAWAY, *Virginia Agricultural Experiment Station*

P52 The Effects of Partial Replacement of Alfalfa Hay with Concentrate on Milk Production. K. A. KENDALL AND R. D. ENGBERSON, *University of Illinois*

P53 Efficiency of Silage and Extra Grain Feeding for Maintaining Summer Milk Production. DWIGHT M. SEATH AND RALPH F. ELLIOTT, *Kentucky Agricultural Experiment Station*

P54 Rates of Grain Feeding for Dairy Heifers on Temporary and Permanent Winter Pasture. S. H. MORRISON AND J. F. DEAL, *University of Georgia*

P55 Kudzu and Fescue-ladino Clover Silage for Dairy Cows. W. A. KING AND J. P. LAMASTER, *South Carolina Agricultural Experiment Station*

P56 Farm Grains vs. a Medium Protein Concentrate Mixture for Cows. K. E. GARDNER, *University of Illinois*

P57 The Relative Palatability of Expeller and Extracted Linseed Meal in Dairy Cow Rations. N. N. ALLEN, *University of Wisconsin*

9:00–11:00 Section B. **REPRODUCTION.** L. O. GILMORE, *Chairman*
*Room 25, Warren Hall*

P58 Preliminary Observation on the Effects of Nutrition on the Quality and Quantity of Bovine Semen. H. H. OLSON, W. E. PETERSEN, T. W. GULLICKSON AND J. N. CUMMINGS, *University of Minnesota*

P59 A Preliminary Report on the Effect of the Site of Semen Deposition on Fertility in Artificial Insemination. G. W. SALISBURY AND N. L. VANDEMARK, *University of Illinois*

P60 Spermatozoan Transport in the Reproductive Tract of the Cow. N. L. VANDEMARK AND A. N. MOELLER, *University of Illinois*

P61 The Effect of Sterile Copulation on the Time of Ovulation in Dairy Heifers. GERMAIN B. MARION, VEARL R. SMITH, THOMAS E. WILEY AND GEORGE R. BARRETT, *University of Wisconsin*

P62 Measuring Reproductive Efficiency in Dairy Cattle. F. A. BUSCHNER, R. E. JOHNSON, C. I. BLISS AND A. A. SPIELMAN, *University of Connecticut*

P63 The Sex Ratio in Calves Resulting from Artificial Insemination. K. E. GARDNER, *University of Illinois*

P64 Artificial Breeding in Alaska and the Effect of Extra Light During the Short Winter Days. WILLIAM J. SWEETMAN, *Alaska Experiment Station*

11:00–12:00 **PRODUCTION SECTION BUSINESS MEETING,** *Room 25, Warren Hall*

*Thursday, June 22*

Afternoon Session

1:30– 3:00 Section A. **MANAGEMENT.** G. M. CAIRNS, *Chairman*
*Room 100, Caldwell Hall*

P65 Nutritive Value of Crops and Cows' Milk as Affected by Soil Fertility. II. The Amino Acid Composition of Colostra and Milk. C. W. DUNCAN, K. M. DUNN AND GERTRUDE I. WATSON, *Michigan Agricultural Experiment Station*

P66 Dairy Cow Stall Studies. I. D. PORTERFIELD, GEORGE HYATT, JR., D. P. BROWN AND A. D. LONGHOUSE, *West Virginia University*

P67 Calf Losses from Disease. H. P. DAVIS, *University of Nebraska*

P68 Relation of Production Records of Cows and Efficiency Management of the Dairy Farm. LEO R. FRYMAN, *University of Illinois*

P69 Relation of Gestation to Body Weights of Cows on Long-time Feeding Trials. R. B. BECKER, P. T. DIX ARNOLD AND SIDNEY P. MARSHALL, *Florida Agricultural Experiment Station*

P70 The Influence of Oestrum on Weights of Holstein and Jersey Heifers. H. B. MORRISON, *Kentucky Agricultural Experiment Station*

1:30– 3:00 Section B. **SEMEN TECHNIQUES.** L. O. GILMORE, *Chairman*

*Room 25, Warren Hall*

P71 The Effect of a Combination of Penicillin, Streptomycin and Sulfanilamide upon the Fertility of Bull Semen. J. O. ALMQUIST AND P. W. PRINCE, *Pennsylvania State College*

P72 Bull Semen Toxicity of Various Salts, Brands and Lots of Penicillin, Streptomycin, Aureomycin and Chloromycetin. JAMES G. SYKES AND JOHN P. MIXNER, *New Jersey Agricultural Experiment Station*

P73 The Influence of Aureomycin upon the Livability and Bacterial Content of Bull Semen. R. M. MYERS, J. O. ALMQUIST AND P. W. PRINCE, *Pennsylvania State College*

P74 Hyaluronidase and Fertility of Dairy Bull Semen. JAMES E. JOHNSTON AND JOHN P. MIXNER, *New Jersey Agricultural Experiment Station*

P75 A Comparison of Two Streptomycin Compounds Used in Diluted Bull Semen. H. L. EASTERBROOKS, P. HELLER, W. N. PLASTRIDGE, E. L. JUNGHERR AND F. I. ELLIOTT, *University of Connecticut*

P76 Results of Breeding Dairy Cows with Egg Yolk Citrate and Ortho Semen Diluters. VICTOR HURST, *South Carolina Agricultural Experiment Station*

## PROGRAM OF EXTENSION SECTION

*Tuesday, June 20*

Afternoon Session. *Room 125, Warren Hall*

1:30– 4:30 **OPENING BUSINESS SESSION AND TEACHING METHODS AND EXHIBITS.** C. W. REAVES, *Chairman*

Address: Effects of Extension in New York. L. R. SIMONS, *Director of Extension, Cornell University*

E1 Master Package for Integrated County Meetings on Dairy Farm Management. S. N. GAUNT, *Massachusetts State College*

E2 Dairy Sub-committee Work in the County Agricultural Planning Program. A. LEON HOLLEY, *University of Arkansas*

Report of Teaching Methods Committee

Explanation and Discussion of Exhibits. JOHN W. FOSTER, *University of Kentucky*, in charge

(Exhibits in *Room 101, Warren Hall*)

*Wednesday, June 21*

Morning Session. *Room 125, Warren Hall*

9:00–12:00 **DAIRY HERD IMPROVEMENT ASSOCIATIONS AND DAIRY CATTLE BREEDING.** RAY ALBRECTSON, *Chairman*

E3 Symposium on DHIA Organization, Operation and Record Analysis by Dairy Records Committee. R. G. CONNELLY, *Committee Chairman*

A. Selection, Training and Orientation of DHIA Personnel. C. R. GEARHART, *Pennsylvania State College*, AND ASSOCIATES

B. DHIA Record Analyses—

*Month to Month Analyses.* D. L. VOELKER, *Iowa State College*, AND ASSOCIATES

*Annual Analyses.* J. F. KENDRICK, *Bureau of Dairy Industry;* L. H. STINNETT, *Oklahoma A. and M. College*, AND ASSOCIATES

C. Organization and Operation of DHIA's. J. D. BURKE, *Cornell University*, AND ASSOCIATES

E4 A Comparison of Monthly, Bi-monthly, and Quarterly Tests for Estimating Milk and Butterfat Production of Dairy Cattle. J. E. STALLARD, *University of Wisconsin*

Report of Dairy Records Committee

E5 The Nature of Reproductive Failures in Dairy Cattle. Illustrated. T. Y. TANABE, *Pennsylvania State College*

*Wednesday, June 21*

Afternoon Session. *Bailey Hall*

1:30– 4:00 **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS AND JOINT COMMITTEE REPORTS.** (See Production Section Program)

4:00– 5:00 **COMMITTEE AND BUSINESS MEETINGS**

*Thursday, June 22*

Morning Session. *Room 125, Warren Hall*

9:00–12:00 **4-H CLUB WORK, COMMITTEE REPORTS, BUSINESS MEETING.**

C. W. REAVES, *Chairman*

E6 Methods Used in Promoting the Junior Dairy Project. GLEN W. VERGERONT, *University of Wisconsin*

E7 How the American Dairy Science Association and the U. S. Department of Agriculture Can Aid in the Development of 4-H Dairy Club Plans and Program. E. W. AITON

E8 Present Dairy Achievement Day and Dairy 4-H Show Ring Activities and How They Can Be Improved. L. A. HIGGINS, *Mississippi*

E9 Judging Contests, Their Development and Limitations. H. R. SEARLES, *Minnesota*

E10 Exhibit of Materials Used in Training 4-H Club Members and 4-H Club Leaders. RALPH PORTERFIELD, *Maryland*

Report of 4-H Club Committee. M. J. REGAN, *Committee Chairman*

**COMMITTEE REPORTS**

**BUSINESS MEETING**

*Thursday, June 22*

Afternoon Session. *Room 125, Warren Hall*

1:30– 3:00 **DAIRY CATTLE HEALTH.** C. W. REAVES, *Chairman*

E11 Hyperkeratosis. PETER OLAFSON, *Cornell University*

E12 Methods of Conducting an Educational Program on Area Brucellosis Control. E. C. SCHEIDENHELM, *Rutgers University*

E13 Dairy Stable Ventilation for Mastitis Prevention. JOSEPH C. NAGEOTTE, *Pennsylvania State College*

E14 Bacteria in the Cow's Udder Associated With Mastitis. JAMES J. REID, *Pennsylvania State College*

E15 Control of Coccidiosis in Dairy Cattle. READ L. DAVIS, *Federal Regional Disease Laboratory, Alabama*

VOLUME XXXIII MAY, 1950 NUMBER 5

# JOURNAL OF DAIRY SCIENCE

## ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

### BOOK REVIEWS

**325. The Market Milk Industry.** C. L ROADHOUSE AND J. L. HENDERSON. 2nd ed. McGraw Hill Book Co., Inc., New York, N. Y. 716 pp. $7.00. 1950.

This widely used text seems to have been thoroughly revised to incorporate postwar changes in methods and added knowledge in the field. Bulk collection of milk from the farm which has been going on for some time in the authors' state, California, is treated in some detail. More space is given to the STHT system of pasteurization, newer cleaning and sterilizing materials, homogenized milk and several newer products such as frozen milk and cream, plastic cream, hydrated milk, recombined milk and yoghurt.

The volume is a complete treatise on the market milk industry. Farm production conditions, especially as they affect the esthetic and sanitary quality of the milk, are well covered. Besides milk plant construction and operation, both distribution and pricing plans show the effects of recent thinking. The portion on the milk plant laboratory and its operation should prove helpful to every person concerned with laboratory control.

The volume contains 171 illustrations most of which readers will recognize as new. The index covers 25 pages. E. F. Goss

**326. Manual for the Cheese Industry.** J. C. MARQUARDT, Marquardt Publishing Co., Geneva, N. Y. 91 pp. $3.00.

This manual is a compact booklet which briefly outlines making procedures for such types of cheese as American, cottage and cream, as well as process cheese, process cheese food and process cheese spreads. No attempt is made to discuss the fundamentals of cheesemaking.

Very little information is given on the difficulties normally encountered in cheesemaking or methods of solving the more common types of manufacturing problems. Brief discussions on milk composition, plant sanitation, starter making and calculations are included. The author states that it was his aim to condense the manual so that facts of immediate concern with better cheesemaking are at hand. This manual will be of particular interest to those desiring a "thumb nail" sketch of the cheese industry and those wanting a quick, handy reference to the more common types of making procedures. H. E. Calbert

**327. Industrial Microbiology.** 2nd ed. S. C. PRESCOTT AND C. G. DUNN. McGraw-Hill Book Co., Inc., New York, N. Y. xii + 923 pp. $8.50. 1949.

Considerable revision and expansion has been incorporated in this new edition. The treatment of the yeasts (primarily those of industrial importance for carbohydrate utilization) has been enlarged and rearranged. Considerable information on newer fermentations has been added. Material on utilization of whey for production of ethyl alcohol has been incorporated. Five new chapters, including one on antibiotics, have been added. Most of the material on lactic acid fermentations, fermented milks, cheese and other food products remains unchanged from the first edition. F. E. Nelson

### ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

**328. The brucella ring test in mixed raw milk supplies.** H. E. BREMER, Vt. Dept. Agr., Montpelier, Vt. Am. J. Pub. Health, **40,** 3: 290–292. Mar., 1950.

Composite samples were taken from the producers' milk cans to avoid vat contamination. Where the herd milk showed a positive or suspicious reaction with the ring test, a blood test on the individuals in the herd would reveal positive or suspicious animals. Complete comparisons could not be made since not all the herd showing such positive or suspicious ring tests were blood tested. The author concludes that the ring test is of value in helping to improve the safety of the raw milk supply and since it is much less expensive than the blood test, it can be applied more often to detect early herd infection.

D. D. Deane

**329. The possibility of disease-free herds.** J. C. Buxton. J. Soc. Dairy Technol., **2,** 4: 201–207. July, 1949.

The general methods of detecting and controlling diseases of cattle are discussed. Emphasis is placed on brucellosis, tuberculosis, Johne's disease and mastitis. E. M. Foster

**330. Method and article for treatment of mammary glands.** F. E. Martin. U. S. Patent 2,498,374. 10 claims. Feb. 21, 1950. Official Gaz. U. S. Pat. Office, **631,** 3: 818. 1950.

For treating mastitis, an antibiotic-containing material which is rigid at normal temperature is dispersed into the teat canal by being forced out of a tube-like device which is inserted in the teat opening. R. Whitaker

## BUTTER

### O. F. HUNZIKER, SECTION EDITOR

**331. Aluminum foil wraps for print butter.** A. H. White. Can. Dairy Ice Cream J., **29,** 2: 46–52. Feb., 1950.

The new foil wrapper which has been developed in Canada and United States consists of a thin 0.0045″ aluminum foil laminated to a lightweight parchment of 15 lb./ream. In some cases, the parchment has been of 27 lb. weight. The foil usually is treated with a protective coating to prevent corrosion from brine. The aluminum foil wrapper combines the good characteristics of vegetable parchment with the added protective qualities of the impermeability of aluminum foil to water, vapor, odors and light. Results indicate that the foil wrapper proved to be much superior to parchment alone in preventing deterioration of the surfaces of prints, absorption of foreign odors and flavors, action of sunlight, loss of weight and color changes at the surface. Some disadvantages of the foil wrap are (a) extra cost; (b) the sharp edge of the foil may cause cuts when butter is wrapped by hand; (c) the foil may become wrinkled and lose some of its attractiveness; (d) foil does not give as much physical protection to butter as parchment plus a waxed carton.
H. Pyenson

**332. Sediment testing of cream and butter.** J. D. Ingle, Swift and Co., Chicago. Natl. Butter Cheese J., **41,** 2: 28–29. Feb., 1950.

The ideal method of making the sediment test is one that will work on all types of cream, will not impair the quality of the cream or alter its churning properties due to added material and will be rapid enough so that it does not delay factory operations.

The condition of the protein in sour cream makes sediment testing of this product difficult. Field tests showed that use of a 10% solution of sodium citrate or a 3% solution of phosphoric acid was most effective in conditioning the cream so that pt. samples could be filtered without undue delay.

Compared with cream, the sediment testing of butter is relatively simple. Seven-cm. filter paper is recommended for laboratory use when a careful microscopic examination of butter sediment is to be made. H. E. Calbert

**333. Butter package.** C. G. Bennett. (Assignor to Paterson Pacific Parchment Co.). U. S. Patent 2,497,203. 2 claims. Feb. 14, 1950. Official Gaz. U. S. Pat. Office, **631,** 2: 418. 1950.

A sheet of moisture-proof material and a sheet of water-permeable material, laminated together with a layer of mold-retarding substance, such as Na or Ca propionate with salt, between the sheets, is used as a wrapper for butter. The moisture-proof side of the wrapper is on the outside.
R. Whitaker

## CHEESE

### A. C. DAHLBERG, SECTION EDITOR

**334. Present-day methods of Stilton cheese manufacture.** T. J. Brindley. J. Soc. Dairy Technol., **3,** 1: 13–15. Oct., 1949.

Two methods used to make Stilton cheese are described briefly. In the method used in some of the larger factories the curd is cut in 0.5-in. cubes and allowed to remain undisturbed in the vat for about 4 hr. After the whey is drained off, the curd is ladled onto perforated drainers in sinks, allowed to settle and cut into 4-in. blocks. The next morning the curd is broken apart, milled, salted and filled into perforated hoops. The cheeses are turned at intervals for about 5 or 6 d. to facilitate even drainage, after which they are moved to a cool, well ventilated room to dry the surface. The cheeses are pierced with needles at weekly intervals for 3 wk. beginning at about 8 wk. after manufacture. Inoculation of the curd with mold spores is left to chance.

The method in general use is essentially similar to that described above except in handling the curd. In this method the curd is not cut but is ladled soon after its formation into coarse linen cloths supported on laths or wires. As drainage progresses the cloths are tied tighter and tighter until the curd is sufficiently firm to transfer onto drainers, after which it is handled as described above. E. M. Foster

**335. History of the Stilton cheese industry.** J. G. W. Stafford. J. Soc. Dairy Technol., **3**, 1: 11–13. Oct., 1949.

The origin of Stilton cheese is described.

E. M. Foster

Also see abs. no. 326, 327, 343, 352.

## CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

**336. Vacreator as a milk evaporating unit.** R. W. Brown and J. J. Janzen. Can. Dairy Ice Cream J., **29**, 1: 56–62, 76. Jan., 1950.

The vacreator has been used as an evaporating unit at the Dairy Science Department at the University of Manitoba during the summers of 1946, 1947 and 1948. 261,131 lb. of skim milk and buttermilk were evaporated at approximately a 3.12:1 ratio. The product was circulated through the vacreator at 160–165° F. By concentrating the product to 24.0% milk solids instead of to 28.0%, the loss of solids in the processing was reduced by an average of more than 50%. The net return per 100 lb. of skim milk and buttermilk amounted to over $1.00 or approximately 5 times the usual value placed on these products. The vacreator-produced skim milk and skim milk and buttermilk mixtures were found to be preferable to skim milk powder of unspecified characteristics when used in ice cream mixes.

H. Pyenson

## DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

**337. The incidence of coliform and milk souring organisms in Welsh farm and creamery water supplies.** Patricia M. Franklin and Gwyneth George. J. Soc. Dairy Technol., **2**, 4: 220–222. July, 1949.

Examination of a large number of dairy water supplies during the past 20 yr. showed that 82% of the farm dairy water samples, 55% of the country creamery samples and 28% of the town dairy samples contained more than 2 coliform bacteria/100 ml. Organisms capable of producing an acid curd in litmus milk incubated at 30° C. for 48 hr. were found in practically all samples that contained coliform bacteria and in some samples that lacked coliform organisms.

E. M. Foster

**338. A note on the use of the colony count at 22° C. in assessing the suitability of a water supply for dairy purposes.** G. E. Jones. J. Soc. Dairy Technol., **2**, 4: 222–223. July, 1949.

The absence of coliform bacteria does not guarantee the suitability of a water supply for dairy use. The author suggests inclusion of a plate count in the bacteriological examination of such water supplies to detect undesirable organisms other than coliform bacteria. He suggests tentatively the use of standard nutrient agar as the medium with incubation for 3 d. at 22° C.

E. M. Foster

**339. The types of bacteria commonly found in farm and creamery water supplies and their action on milk and milk products.** S. B. Thomas. J. Soc. Dairy Technol., **2**, 4: 224–232. July, 1949.

The author presents a general discussion of the types of bacteria likely to be found in water supplies and the effects they may cause based on his study of 242 farm water supplies; it is supplemented generously by reference to pertinent published articles. The danger of using untreated water for washing dairy utensils, plant equipment, butter, etc. is stressed particularly from the standpoint of the introduction of spoilage organisms.

E. M. Foster

**340. Bacteriological colony counting device having a light-conducting member for transverse illumination.** R. M. Wood. U. S. Patent 2,495,-912. 6 claims. Jan. 31, 1950. Official Gaz. U. S. Pat. Office, **630**, 5: 1230. 1950.

To facilitate counting the colonies on a petri plate, this counter is so designed that light from a lamp is reflected from the polished inside wall of a globular-shaped bowl beneath the plate and illuminates the colonies transversely from all sides. A mask under the dish prevents direct rays of light from shining through the dish from beneath.

R. Whitaker

**341. The "5-minute" resazurin test for determining the quality of raw milk.** J. C. Boyd and H. C. Hansen, Univ. of Idaho, Moscow. J. Milk and Food Technol., **13**, 1: 40–43. Jan.-Feb., 1950.

The resazurin test using 5, 10 and 15 min. periods was compared to the methylene blue test. The results showed the 5-min. reading to be the most accurate in grading poor milk. Resazurin "B" grade milk in 5 min. showed 71.9% of the samples had direct microscopic counts ranging from 21,000,000 to 60,000,000/ml.

The authors suggest that the resazurin test should be supplemented by other tests, preferably the direct microscopic count. The "5-min." resazurin test is valuable when the milk will reduce methylene blue in 3 hr. or less.

H. H. Weiser

**342. Preliminary observations on the application of an alcohol screening test, used in con-**

**junction with the ten-minute resazurin test, for detecting unsatisfactory milk on arrival at creameries.** ANN E. HUGHES AND DOROTHY ELLISON, National Milk Testing Service, Brynawel, Aberystwyth. J. Soc. Dairy Technol., **2,** 3: 149–151. Apr., 1949.

Alcohol strengths of 76, 72 and 68% were compared with the 10-min. resazurin test for detecting poor quality milk in 378 samples tested during the summer of 1948. Relatively good agreement was obtained between the alcohol tests and the samples showing good quality by the resazurin test. However, 27% of the samples showing poor quality by the resazurin test were not detected by the 76% alcohol, and an even greater percentage was missed by the lower alcohol concentrations. The authors feel that even though a high proportion of poor quality milks is missed by the 76% alcohol test it is still more reliable than visual and olfactory examination by a plant worker. E. M. Foster

**343. The bacteriophage of cheese cultures.** C. E. PARMELEE, F. E. NELSON, G. E. TURNER AND P. H. CARR. Iowa Agr. Expt. Sta., Ames. Natl. Butter Cheese J., **41,** 1: 28–30. Jan., 1950.

Acid production virtually is stopped in single strain cultures when they are attacked by bacteriophage. In mixed cultures an attack by bacteriophage is characterized by slow acid production. Bacteriophage particles cannot be seen with the ordinary microscope. By use of the electron microscope they appear to be sperm-shaped, 220 $m\mu$ long, with a head 70 $m\mu$ in diameter. The tail is approximately 150 $m\mu$ long and 30 $m\mu$ wide.

A simple method of determining relative numbers of bacteriophage particles in an infected material is to add a measured amount of susceptible culture plus a measured amount of properly diluted material to tubes of sterile skimmilk. Incubate tubes 12–14 hr. at 32° C. Under these conditions the control containing only organisms will be coagulated. The presence of bacteriophage is indicated when there is no coagulation in the tubes of lower dilution. The amount of bacteriophage particles present in the suspected material can be judged by the highest dilution that evidences bacteriophage activity.

Another method for determining relative numbers of bacteriophage particles depends on the production of clear areas or plaques on agar plates. There are several illustrations of this method. H. E. Calbert

**344. The occurrence of Proteus spp. in raw and pasteurized milk.** A. J. ZARETT, R. N. DOETSCH AND P. ARNE HANSEN. Univ. of Md., College Park. J. Milk and Food Technol., **13,** 1: 31–34. Jan.-Feb., 1950.

The frequency of the *Proteus* group of organisms in the intestinal tract has been reported by several investigators. Obviously milk carelessly handled likely will contain human and animal excreta. It has been suggested that *Proteus* serve as a test organism to indicate pollution in milk instead of the *Escherichia-Aerobacter* group. The use of *Proteus* would have the added advantage in that no species differentiation would be necessary, since each species would have some sanitary significance. The use of urea-recinoleate agar culture medium to detect *Proteus* colonies was satisfactory and necessitated no further biochemical tests. H. H. Weiser

**345. Pyridoxal phosphate and pyridoxamine phosphate as growth factors for lactic acid bacteria.** W. S. MCNUTT AND E. E. SNELL, Univ. of Wis., Madison. J. Biol. Chem., **182,** 2: 557–567. Feb., 1950.

In a medium shown to be complete for the growth of most lactic acid bacteria, 1 species each of *Lactobacillus helveticus, L. acidophilus* and *L. delbrueckii* grew only when such natural materials as malt sprouts or autolyzed yeast were included in the medium. Observations made during the fractionation and concentration of malt sprout extract led to tests of the growth-promoting effect of phosphate esters of light-sensitive vitamins. For maximum growth of *L. helveticus* and *L. acidophilus,* 1 $m\gamma$ of pyridoxamine phosphate/ml. medium is equivalent to 200$\gamma$ malt sprouts provided any one of the following substances was included: non-specific reducing agents, vitamin $B_{12}$ and thymidine or other desoxyriboside. A 7-fold concentration of pyridoxal phosphate could substitute for pyridoxamine phosphate; both vitamin $B_6$ phosphates could be replaced by D-alanine.

Except for a specific requirement for thymidine in these experiments, the strain of *L. delbrueckii* responded similarly to phosphorylated vitamin $B_6$ and D-alanine.

Pyridoxamine, phyridoxal and L-alanine were inactive at the concentrations tested. Coenzyme I and II, flavin-adenine dinucleotide, cocarboxylase and various nucleotides exhibited very low activity or were inactive. H. J. Peppler

**346. Competitive antagonism of ribonucleic and desoxyribonucleic acids in the nutrition of Lactobacillus bifidus.** H. R. SKEGGS, J. SPIZIZEN AND L. E. WRIGHT, Sharpe and Dohme, Inc., Glenolden, Pa. J. Am. Chem. Soc., **72,** 2: 811–813. Feb., 1950.

*Lactobacillus bifidus* (ATCC4963), recently reclassified as *L. acidophilus,* is capable of utilizing

either thymidine or vitamin $B_{12}$ for growth in an otherwise complete medium. In the absence of either of these substances, this organism responds to increasing amounts of intact desoxyribonucleic acid (DNA) over a range of 0.5 to 5γ/ml. This lactobacillus is more sensitive to DNA as an essential nutrient than to vitamin $B_{12}$. Ribonucleic acid (RNA) competitively inhibits the utilization of DNA by *L. acidophilus*. In view of the essential nature and widespread occurrence of DNA in all living cells, the specific blocking of DNA utilization by RNA could be of great importance in many studies of cellular metabolism.

H. J. Peppler

Also see abs. no. 327, 328, 365, 384.

## DAIRY CHEMISTRY

### H. H. SOMMER, SECTION EDITOR

**347. Isolation of DDT from fats.** B. Davidow, Food and Drug Administration, Federal Security Agency, Washington 25, D. C. J. Assoc. Off. Agr. Chemists, **33,** 1: 130–132. 1950.

The conventional Schechter-Haller colorimetric method for determination of DDT was not applicable in the presence of more than traces of fat, and the isolation of DDT from large quantities of fat has remained a serious analytical problem.

The author outlines in detail a more convenient and faster method for the separation of fat from DDT. Celite (commercial diatomaceous earth) impregnated with sulfuric acid-fuming sulfuric acid and slurried with carbon tetrachloride holds fats within a chromatographic column while DDT paced through with the carbon tetrachloride. Recoveries of microgram quantities of DDT from 5 g. of butteroil ranged from 90 to 100%.

F. J. Babel

**348. Effect of thymus nucleate on the thermal coagulation of albumin solutions.** J. P. Greenstein and M. L. Hoyer, Nat. Cancer Inst., Bethesda, Md. J. Biol. Chem., **182,** 2: 457–466. Feb., 1950.

The coagulation of bovine and horse serum albumin and chicken egg albumin in aqueous solutions (pH 6.4–6.7) was prevented by relatively small amounts of sodium thymus nucleate provided both albumin and nucleate solutions are practically salt-free. One mg. of nucleate nearly completely protects about 150 mg. albumin against coagulation at 98° C. for 10 min. The degree of coagulation of the heated protein was nearly linear with decreasing amounts of nucleate. Yeast nucleate has no apparent effect on albumin, even at relatively high concentrations. Unlike other agents known to increase the thermal stability of albumin, thymus nucleate does not prevent denaturation on heating, but only coagulation. When heated solutions of albumin and protecting nucleate were treated with desoxyribonuclease and magnesium ions, coagulation of the heated protein gradually ensued as the protecting nucleate was digested. H. J. Peppler

**349. Manufacture of lactalbumin.** E. C. Scott and G. W. McDonald (Assignors to Swift and Co.). U. S. Patent 2,497,420. 2 claims. Feb. 14, 1950. Official Gaz. U. S. Pat. Office, **631,** 2: 473. 1950.

The acidity of whey is adjusted to between 0.07 and 0.12% calculated as lactic acid. It then is heated to a temperature at which the albumin normally coagulates, but does not under these conditions. With minimum agitation there is added to the quiescent heated whey additional acid to bring the whey to 0.15–0.3% calculated as lactic acid. This causes the lactalbumin to precipitate suddenly in large floating lumps, from which the whey readily is drained. R. Whitaker

**350. The sedimentation constant, diffusion constant and molecular weight of lactoglobulin.** R. Cecil and A. G. Ogston, Univ. of Oxford. Biochem, J., **44,** 1: 33–35. 1949.

Sedimentation and diffusion constants were made on 3 preparations of lactoglobulin and from these values a molecular weight of 35,400 was calculated. This figure is in good agreement with other reported values. There is some discussion regarding differences observed.

A. O. Call

**351. Physics in the dairy industry.** R. Harper, Nat. Inst. for Research in Dairying, Univ. Reading, England. J. Soc. Dairy Technol., **3,** 1: 39–45. Oct., 1949.

The physical characteristics of milk, cream, butter and cheese are discussed. Normal values for viscosity, density, size of fat globules, freezing point, refractive index and surface tension are given for milk and, in some cases, for cream.

E. M. Foster

## DAIRY ENGINEERING

### A. W. FARRALL, SECTION EDITOR

**352. Apparatus for agitating curds.** P. P. Caumartin. U. S. Patent 2,496,001. 3 claims. Jan. 31, 1950. Official Gaz. U. S. Pat. Office, **630,** 5: 1253. 1950.

This agitator has 2 flanged rollers which roll along the top of the sides of the cheese vat and support an arm which extends down into the vat.

Attached to the lower end of the arm is a paddle wheel-shaped curd agitator, driven by a chain and sprocket from a power source above the vat. R. Whitaker

**353. Instrumentation for high temperature short time pasteurization.** W. S. Young, The Foxboro Co., Foxboro, Mass. Am. Milk. Rev., **11,** 10: 26–29, 50, 51. Oct., 1949.

Instruments for high-temperature, short-time pasteurization include heater temperature controller, flow diversion valve operator, cold milk recorder and brine temperature controller. An electronic timer for determining holding time by timing a heat impulse is described. The design and mechanics of operation of the flow diversion valve are explained. D. J. Hankinson

**354. Routine maintenance of short time high temperature control systems.** R. E. Olson, Taylor Instrument Co, Rochester, N. Y. Am. Milk Rev., **11,** 10: 33, 57. Oct., 1949.

Periodic checking of high-temperature, short-time equipment should include (a) cleaning air valve in pressure controller, (b) blowing out all air filters daily, (c) cleaning air valve in temperature controller, (d) checking accuracy of safety thermal limit recorder, (e) examining rubber disc rings in flow diversion valve for wear and leakage and (f) examining all air pipe connections for leaks.

The cause and correction of the following troubles are indicated: (a) Lag in reaching fixed milk temperature, (b) wandering of final milk temperature, (c) continuous "hunting" of final milk temperature, (d) failure of flow diversion valve to operate when milk is at correct temperature, (e) failure of flow diversion valve to remain in forward flow position and (f) failure of milk pump to start. D. J. Hankinson

**355. Refrigeration in manufacture of ice cream.** H. M. Tyrrell. Can. Dairy Ice Cream J., **29,** 1: 27–29, 74. Jan., 1950.

In order to have sufficient compressor capacity, one should know what refrigeration is required at the temperature that one wishes to operate. For example, a 10-ton machine will do 10 tons only at 19.6 lb. back pressure. About 10% of freezer troubles are due to refrigeration. New freezers should not be piped up to existing suction and liquid lines to the compressor as the lines usually are too small to take care of the additional load. The installation of an ammonia still is an effective way of cleaning the system of oil and "dead" liquid. In the hardening room, ice cream hardens in about half the time when properly stacked and when proper blast fans are used rather than still air. The proper oil should be selected for the ammonia compressor for the temperature range at which the compressor is to operate. Brine and brine systems should be kept at pH 9 and at the proper specific gravity to give the necessary freezing point. Sodium chromate inhibits corrosion in brines. In all plants operating on a low back pressure, it is necessary to install an automatic purger. H. Pyenson

**356. Dairy refrigeration.** F. N. Beams. J. Soc. Dairy Technol., **2,** 2: 115–121. Jan., 1949.

The basic principles of refrigeration are described briefly along with some of the common types of refrigerating systems. The application of refrigerating equipment to cooling and storage of fluid milk and ice cream are discussed. E. M. Foster

**357. Standards have to grow.** F. A. Faust, The Bristol Co., Waterbury Conn. Am. Milk Rev., **11,** 10: 42, 43. Oct., 1949.

The 3A standards have been established which have eliminated threaded construction on instrument fittings in contact with milk. Other subjects under investigation include homogenizer flange, cold milk recorder specifications, storage tank sockets, "blister bulb" construction for storage tank controllers, specifications for high-temperature, short-time pasteurizers and thermal timing methods for determining holding time for high-temperature, short-time equipment. D. J. Hankinson

**358. Electronic instruments for dairy processors.** J. Meyer, Minneapolis-Honeywell Regulator Co., Philadelphia, Pa. Am. Milk Rev., **11,** 10: 34, 49. Oct., 1949.

The applications of the pyrometer (thermocouple plus potentiometer) are discussed. Multipoint recorders offer certain advantages for control work. Such electronic equipment offers an obtainable accuracy of 0.125° F. and is difficult to break and easy to fix. Instruments not now available may be offered to the industry in the future. D. J. Hankinson

**359. Possibilities in the sterilization of milk by means of radiations.** H. Burton. J. Soc. Dairy Technol., **2,** 2: 75–80. Jan., 1949.

The possible applications of radio frequency waves, ultraviolet rays, X-rays, gamma rays, cathode rays, alpha particles, neutrons and sonic and ultrasonic waves in the treatment of milk are discussed with references to pertinent literature. The author believes that of the methods listed only ultraviolet radiation seems to be approaching the stage where commercial utilization is possible. E. M. Foster

**360. Method of sterilizing and preserving.** A. BRASCH (Assignor to Electronized Chemicals Corp.). U. S. Patent Reissue 23,195. 17 claims. Feb. 7, 1950. Official Gaz. U. S. Pat. Office, **631,** 1: 79. 1950.

Reissue of U. S. Patent 2,456,909 covering sterilization of milk and other foods by bombardment with high speed electrons of a velocity of 1 million volts for not over 0.000001 sec.

R. Whitaker

**361. Temperature and pressure instruments in the dairy industry.** A. LEBOUTILLIER, Taylor Instrument Co., Rochester, N. Y. Am. Milk Rev., **11,** 10: 8–10, 55. Oct., 1949.

The Bourdon tube as a temperature and pressure measuring device is described. Various temperature control systems are briefly discussed, including on-off control, proportional response, automatic reset and the self-acting regulator. The application of these control systems to the short-time, high-temperature pasteurizer is explained.

D. J. Hankinson

**362. Bourdon pressure spring.** J. W. BEECHER, The Bristol Co., Waterbury, Conn. Am. Milk Rev., **11,** 10: 12, 56. Oct., 1949.

The design and manufacture of Bourdon pressure spring for use in pressure and temperature measuring instruments is discussed.

D. J. Hankinson

Also see abs. no. 336.

## DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

**363. Waste prevention in the dairy industry.** E. F. ELDRIDGE. Milk Dealer, **39,** 5: 49, 50, 88–94. Feb., 1950.

This is a report of the Task Committee on Dairy Waste Disposal of the Dairy Industry Committee. The average stream contains from 7–10 parts of oxygen per million parts of water. Wastes from dairy plants deplete this oxygen until streams cannot maintain normal aquatic life and thus, unpleasant odors, black sludge deposits and gray fungus growth result. The oxygen from 1,600 gal. or normal, unpolluted stream water is required to decompose the organic material in 1 pt. of milk. The average milk receiving station with good housekeeping methods can keep its milk waste to limits of 4 lb. of biological oxygen demand /10,000 lb. of milk, or a milk loss of 0.35%. The following sources of waste in dairy plants and methods of preventing them are discussed: (a) Leakage, (b) overflow, (c) spillage, (d) freezing on, (e) willful waste, (f) residual waste and (g) carry over. Data is presented showing the approximate average composition of milk and milk products, the BOD and population equivalent per hundredweight. The most important processes in the dairy industry and the pounds BOD loss which can be expected from each process with reasonably modern equipment and careful operation also is shown. A waste prevention program is discussed. C. J. Babcock

**364. Cutting clerical costs.** Anonymous. Milk Dealer, **39,** 4: 43, 70–72. Jan., 1950.

Pet Dairy Products Co. of Greensboro, N. C., has cut clerical costs on accounts receivable by one-half through use of photographic equipment. The advantages of the system are: (a) The customer has a completely detailed, neat invoice. (b) The milk dealer has a full record of each transaction on an easily-indexed, ready reference film roll. (c) Time-and-a-half of clerical effort has been completely freed for other activity. (d) Duplication of effort is materially reduced. (e) Bills are out sooner, and collections are better. (f) Follow-up of inactive accounts is surer and faster.

The microfilm rolls lend themselves to simple but complete indexing; reference to current or old records is fast. The reader, in addition to presenting an image in sharp, clear enlargement also serves as a fast, inexpensive printer, should a copy of the statement, invoice or other filmed document be required. C. J. Babcock

Also see abs. no. 385, 388.

## FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

**365. Preliminary observations upon factors influencing cellulose digestion by rumen microorganisms.** W. BURROUGHS, N. A. FRANK, P. GERLAUGH, AND R. M. BETHKE, Dept. of Animal Industries, Ohio Agr. Expt. Station, Reynoldsburg. J. Nutrition, **40,** 1: 9–24. Jan., 1950.

Cellulose digestion by rumen microorganisms was carried out in 500-ml. glass containers at 40° C. Filter paper was the source of cellulose. The starting inoculum was a mixed culture of organisms taken directly from rumens. $CO_2$ was bubbled through the liquid. Digestion was allowed to proceed for 36 hr. at which time ½ of the fermentation medium was removed, with the remaining amount serving as an inoculum for a succeeding 36-hr. digestion period. The portion removed was analyzed to determine cellulose digestion. Those additions to the fermentation media which favored cellulose digestion were a complex salt solution, ash of alfalfa extract, auto-

claved rumen liquid and autoclaved water extract of manure. R. K. Waugh

**366. Yellow gas from corn silage.** W. H. Peterson, R. W. Thoma and R. F. Anderson, Univ. of Wis. Hoard's Dairyman, **94,** 23: 870–871. Dec. 10, 1949.

Reports of yellow gas, fatal to chickens and flies, around the bottom of a silo were investigated. A sample of such gas was obtained from the silo room at the bottom of the chute of one of the University of Wisconsin silos. The sample was found to contain both nitrites and nitrates. Nitrogen dioxide at the rate of 151 parts per million were found. The yellow gas is believed to come from the reduction of nitrates in the corn. The production of yellow gas stops after a few days. No danger from the use of this silage is expected. A. R. Porter

## GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

**367. Proving sires and dams.** Anonymous. Hoard's Dairyman, **94,** 23: 872–873. Dec. 10, 1949.

The report of a committee of the Purebred Dairy Cattle Association to set up uniform rules for proving sires and dams is given. Seven points in the recommended procedure of proving sires and dams are listed. A preliminary proof on sires is recommended with the first 5 daughter-dam comparisons and a proof should be reported with 10 or more comparisons. The recommended proving of a dam includes weighted values of records of the cow, her daughters and her sons' daughters. A. R. Porter

## HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

**368. Milk flow controls for milking machines.** A. G. Perkins. U. S. Patent 2,496,307. 23 claims. Feb. 7, 1950. Official Gaz. U. S. Pat. Office, **631,** 1: 92. 1950.

A spring-loaded valve is described which controls the milk flow from a vacuum operated milking machine, cutting off the vacuum when the milking operation is complete. R. Whitaker

**369. Supporting means for milking apparatus.** S. Daly (Assignor to International Harvestor Co.). U. S. Patent 2,497,299. 5 claims. Feb. 14, 1950. Official Gaz. U. S. Pat. Office, **631,** 2: 442. 1950.

A stand equipped with rack and pinion allows adjustment of a milk collecting vessel below the udder. The teat cups are attached with relatively short hose connections. R. Whitaker

**370. Milking system and apparatus therefor.** G. R. Duncan. U. S. Patent 2,498,401. 14 claims. Feb. 21, 1950. Official Gaz. U. S. Pat. Office, **631,** 3: 824. 1950.

Milk cans standing in a refrigerated cabinet are filled directly from a milking machine through a device located in the cover of the cans which permits discharge of the milk with no loss of vacuum. R. Whitaker

Also see abs. no. 398.

## ICE CREAM

C. D. DAHLE, SECTION EDITOR

**371. Ice cream—paper containers in relation to shrinkage.** J. A. Meiser, Mich. State College, East Lansing. Sou. Dairy Prod. J., **47,** 3: 30–31. Mar., 1950.

Results of the experiment reported indicate that containers in themselves will not necessarily prevent shrinkage, but certain treatments of the containers may retard the volume loss to some extent. Coating paper containers with paraffin or glassine definitely lessened the degree of shrinkage of ice cream. F. W. Bennett

**372. Formulas for making sherbets with whey on a commercial scale.** F. E. Porter and D. H. Williams, U. S. D. A., Washington, D. C. Ice Cream Trade J., **46,** 2: 52. Feb., 1950; Ice Cream Rev., **33,** 7: 53. Feb., 1950.

See abs. no. 218.

**373. The manufacture of culturized ice cream.** W. H. E. Reid, Univ. of Missouri, Columbia, and C. B. Agee, R. M. Hanckel and R. H. Thomas. Sou. Dairy Products J., **47,** 3: 34, 122–124. Mar., 1950.

The effects of the addition of a dehydrated culture of lactic acid bacteria in de-fatted milk in ice cream were studied. The acidity of the dehydrated culture was standardized to a pH slightly above that of non-fat dry milk solids, prior to dehydration by the addition of a standardizing agent "Minsol."

Dehydrated culture used as a source of serum solids imparted a distinctive culture flavor to ice cream. Mixes containing 3 and 5% of dehydrated culture were superior in smoothness and mellowness of body and closeness of texture. Dehydrated culture showed unusual stabilizing properties and when added at the rate of 5% of the total weight of the mix, tended to cause the resulting ice cream to be too stable. F. W. Bennett

**374. Fruit strainer.** J. B. ORRELL (Assignor to Abbotts Dairies, Inc.). U. S. Patent 2,496,636. 6 claims. Feb. 7, 1950. Official Gaz. U. S. Pat. Office, **631,** 1: 178. 1950.

This device separates the juice from the pulp of fruit for flavoring ice cream, etc. A screw conveyor, operating in a perforated cylinder within a closed inclined cylinder of larger diameter, propels the pulp along to a discharge hole, while the juice escapes through the perforations and is collected between the 2 cylinders and is removed through a bottom drain. R. Whitaker

**375. Cocoa products for ice cream.** L. FREUNDLICH, Hooton Chocolate Co., Newark, N. J. Ice Cream Trade J., **46,** 2: 62, 63, 72. Feb., 1950.

Choice of flavoring material may depend on the price relationship of milk fat and cocoa butter, also on whether the manufacturer has an homogenizer. If no homogenizer is available, it is best to use cocoa, because it is easier to disperse it into a syrup than it is to disperse chocolate liquor. Less stabilizer is recommended for chocolate ice cream than for vanilla, and the higher the fat content of the chocolate flavoring the less stabilizer will be needed.

Cocoa should be free from gritty matter. All of it should pass through a 100 mesh sieve and at least 97% through a 200 mesh sieve. The finer the powder, the better will be its flavor. The numerous varieties of cocoa beans each has its characteristic color and flavoring. Chocolate and cocoa manufacturers blend different cocoas to get the desired color and flavor characteristics for ice cream.

Color intensity in chocolate ice cream may be due to variation in density, manner of dipping shrinkage or change in acidity due to bacterial activity. W. H. Martin

**376. Ice cream disher.** S. BLOOMFIELD. U. S. Patent 2,498,331. 2 claims. Feb. 21, 1950. Official Gaz. U. S. Pat. Office, **631,** 3: 807. 1950.

This is a modification of the well known semicircular ice cream disher with a blade which rotates within the bowl to discharge the ice cream. The novel feature is the ratchet arrangement which rotates the shaft attached to the discharging blade. R. Whitaker

**377. Melvern's "prepacked" low overrun, bulk pint.** Ice Cream Trade J., **46,** 2: 42, 106. Feb., 1950.

Melverns Dairies, Inc., of Washington, D. C., are marketing a factory-filled, pail-type package of ice cream which contains 14% fat and 43% overrun retailing at 35¢/pt. and costing dealers 26¢. All pre-packed bulk pints are sold in solid flavors in specially designed cartons. In order to freeze the ice cream with 43% overrun, it is necessary to reduce the freezer capacity one-third. W. H. Martin

**378. Producing sandwiches in volume with low labor costs.** Anonymous. Ice Cream Trade J., **46,** 2: 66. Feb., 1950.

A potential process developed by LeRoy Foods, Inc., Brooklyn, N. Y., makes possible production of 400 doz. ice cream sandwiches an hour at a cost of about 1¢/doz. Sandwich wafers 2.75 in. square placed in individual special cartons holding 3.5 fluid oz. are used. Units of 24 are filled with ice cream directly from the freezer and go on to the hardening room. W. H. Martin

**379. Recent developments in making ice cream novelties.** E. J. OTKEN, Good Humor Corp., Brooklyn, N. Y. Ice Cream Trade J., **46,** 2: 50. Feb., 1950.

Production of chocolate-coated ice cream stick confection has grown to 3–3.5 billion annually, valued at $300,000,000. Details of current production practices are given. W. H. Martin

**380. The flaming nut sundae.** Anonymous. Ice Cream Trade J., **46,** 2: 46. Feb., 1950.

The flaming nut sundae was introduced by the Liggett-Rexall Drug Store Chain. A pint of vanilla ice cream is cut in half lengthwise and one-half placed in a banana split dish. Hot fudge (1½ oz.) then is streamed lengthwise over the ice cream and 1 tablespoon of pecan bits is sprinkled over this. Half a marshmallow is placed in the center with a cube of sugar freshly moistened with 3 drops of lemon extract on it. The extract, containing about 80% alcohol, burns brightly when lighted. The sundae sells for 29¢. W. H. Martin

**381. 1949 gallonage.** Anonymous. Ice Cream Trade J., **46,** 2: 44, 98. Feb., 1950.

According to figures released by the U. S. D. A., the estimated 1949 production of ice cream was 553,650,000 gal., 3% below the 1948 volume. New York State's production was 61,440,000 gal., an increase of 5 million gal. over that of 1948. Pennsylvania produced 65,380,000 gal., a slight increase over 1948. W. H. Martin

**382. The new national drug store survey.** Ice Cream Trade J., **46,** 2: 74–82. Feb., 1950.

See abs. no. 302.
Also see abs. no. 355, 356, 391.

## MILK AND CREAM

P. H. TRACY, SECTION EDITOR

**383. "Trouble shooting" in quality control work.** W. A. CORDES, National Dairy Products Co., Inc. Milk Dealer, **39,** 4: 41, 102. Jan., 1950.

A discussion is given of quality defects in dairy products which are a result of the following factors: (a) poor quality of raw materials, (b) lack of proper processing equipment to do the job, (c) failure of equipment to do the job for which it is designed, (d) lack of proper facilities and supplies, (e) failure of personnel to use equipment properly to do the best possible job with the facilities available, and (f) failure of management and supervision to supply personnel with directions and formulas and to instruct, supervise and check personnel. The necessity of training and experience is emphasized. C. J. Babcock

**384. Screening and rejection tests in relation to the keeping quality of milk.** ELEANOR JONES-EVANS. J. Soc. Dairy Technol., **2,** 4: 232–236. July, 1949.

A 72% alcohol test and the 10-min. resazurin test were compared on 1,341 samples of milk taken at 9 creameries in mid- and south Wales during April-Sept., 1948. Accuracy of the tests in detecting milk of poor quality was checked by comparing with keeping quality of the samples as determined by holding at 20° C. and performing clot-on-boiling tests at intervals.

The alcohol screening test was judged unreliable because it passed as satisfactory a high proportion of samples with keeping quality times less than 12 hr. Samples judged unsatisfactory by the 10-min. resazurin test (disc numbers 0–3.5) usually had unsatisfactory keeping times (less than 12 hr.). However, samples with higher disc number (4–6) showed a wide range of keeping quality times. It was concluded that disc readings of 4–6 on the 10-min. resazurin test gave little indication of the actual keeping quality of the milk. E. M. Foster

**385. Can the Northeast forget manufactured milk?** E. O. MATHER, Dellwood Dairies, Yonkers, N. Y. Am. Milk Rev., **11,** 11: 2–4, 55. Nov., 1949.

The formula method of determining the price of Class I (fluid) milk in the Boston market is unrelated to the price of milk for manufactured dairy products. The New York Class I-A price is tied to the Boston Class I price. Until 1948, the price of milk for fluid purposes was approximately $1.25/cwt. over the midwestern condensery price. In the 10-mo. period Sept., 1949, through June, 1949, the New York Class I-A price was $2.49/cwt. or almost double the historical price. The problems resulting from this price relationship are (a) increase in milk production causing lowered blend price, (b) increased pressure from outside producers to get into the pool, causing a lowered blend price, (c) very low prices for surplus milk which is in competition with midwestern cream and butter and (d) consumption of evaporated milk encouraged at the expense of fluid milk. Furthermore, with formula pricing of Class I (fluid) milk according to economic conditions and with government support for manufactured dairy products, it is possible that northeastern producers might experience a very low return if economic conditions become serious. D. J. Hankinson

**386. The consumer looks at a bottle of milk.** Mrs. MILDRED MESKIL, N. Y. State Dept. of Commerce, Albany, N. Y. Milk Dealer, **39,** 4: 46, 78–82. Jan., 1950.

A questionnaire distributed to consumers in 3 major up-state New York urban areas reveals that ¾ of all the people interviewed had milk delivered at home and 24.6 of the remaining 25% purchased milk in some form or other, usually from the local store. Most consumers demanded regular delivery and 93% agreed they got it but 22% were not satisfied with the delivery time. There was an overwhelming preference for square glass containers. Fifty-six per cent did not want the 2-qt. bottle and many of those who would use it would expect a price reduction. Sixty-two per cent indicated that they found it desirable to purchase other commodities from the milkman. In the low income group, 60% bought milk because it was good for them, while in the middle income group 78.8% bought for this reason. In the first group, 63.3% used it in cooking; the latter group bettered this figure by 10%. Fifty per cent of all respondents bought because it was good for the children and 45% simply because it tastes good. Three-fourths of all who answered were familiar with homogenized milk; half of these purchased it and of that total 86% prefer it. The only objection to homogenized milk was no "top milk" for coffee or cereal use. Some 98.5% of those replying use fresh milk; 43.6% use evaporated milk; 37.9% use cream; 27.9% use chocolate milk and 1.7% use powdered milk. C. J. Babcock

**387. What do consumers think of bottled fresh concentrated milk?** G. M. TROUT AND G. G. QUANKENBUSH, Michigan State College, East Lansing. Am. Milk Rev., **11,** 11: 42–45, 55. Nov., 1949.

See abs. no. 147.

**388. Milk tokens.** P. G. KEMP. Can. Dairy Ice Cream J., **29,** 1: 33, 71. Jan., 1950.

The cost of milk tokens is only about 25% of the cost of tickets. There is a savings of the cashier's time and the route salesman's time. Customers have accepted tokens about 99.5%. Milk tokens easily drop out of a damp milk bottle. Large stocks of tokens are not needed as they are kept in circulation. On a 10,000 qt. of milk/month basis, only 1,500 milk tokens would be required for each product. Counterfeiting has not yet been a problem. H. Pyenson

Also see abs. no. 325, 341, 342, 344, 357, 359, 360.

## MILK SECRETION

V. R. SMITH, SECTION EDITOR

**389. Some recent researches on milk secretion.** H. D. KAY. J. Soc. Dairy Technol., **3,** 1: 17–21. Oct., 1949.

A discussion of the role of hormones in milk secretion is presented. E. M. Foster

## NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

**390. Food value of milk and dairy products.** E. A. MARTIN. Can. Dairy Ice Cream J., **29,** 2: 66–76, 88. Feb., 1950.

This paper was delivered by the author at the 12th International Dairy Congress, Stockholm, Sweden, 1949. It covers rather completely the food value of milk, butter, cheese, whey and ice cream and means of increasing their consumption through nutritional research projects, printed literature, motion pictures, advertising, convention exhibitis, publicity and leader contacts. H. Pyenson

**391. Ice cream in the field of nutrition.** H. D. BRANION. Can. Dairy Ice Cream J., **29,** 2: 39–41, 90. Feb., 1950.

Ice cream has been shown to be a food and not a confection. Assays and analyses on ice cream from a nutritional angle are few. Ice cream stands out for mineral calcium, riboflavin and vitamin A content. Canadian dietary often lacks calcium and riboflavin and by increasing the consumption of ice cream more would be obtained. H. Pyenson

## PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

**392. The response of the ovary of the anestrous goat to pregnant mares' serum gonadotrophin.** S. J. FOLLEY, A. L. GREENBAUM AND A. ROY, Nat. Inst. for Research in Dairying, Univ. of Reading, England. J. Endocrinol., **6,** 2: 121–131. Oct., 1949.

Goats during the anestrous season were brought into estrous by the injection of pregnant mares' serum (p.m.s.) and subsequently mated. Of 35 goats injected with a single subcutaneous dosage of 1200 i.u. of p.m.s. and brought into heat, only 22.8% produced young following copulation. This low conception rate could be accounted for in part by a lowered fertility of the male during the anestrous season. No differences in response were observed in comparing subcutaneous to intravenous hormone dosage. The dosage used induced super ovulation but no concomittant superfetation was noted. In some cases, estrous did not follow p.m.s. injection although ovulation was known to have occurred. V. Hurst

**393. Influence of variation in environmental temperature and thyroid status on growth and feed consumption of the male mouse.** M. MAQSOOD AND E. P. REINEKE, Mich. State College, East Lansing. Am. J. Physiol., **160,** 2: 253–258. Feb., 1950.

Growing male mice were studied under environmental temperatures of 24 and 30° C. with a relative humidity held between 45–55%. Control mice were fed Purina laboratory chow and experimental mice were fed the chow plus varying amounts of thiouracil or thyroprotein or combinations of thiouracil and thyroprotein. Feed and water consumption were measured.

Control mice at 30° C. gained less weight than control mice at 24° C. Thiouracil feeding depressed growth at both temperatures. A dosage of thyroprotein (0.025%) which stimulated growth at 24° C. caused growth depression at 30° C. Growth stimulation by feeding thyroprotein could be induced at 30° C. but the stimulating dosage had to be reduced to 0.005% of the ration.

Thiouracil feeding lowered both food and water consumption at high and low temperatures. Thyroprotein feeding increased food intake in proportion to dosage even when increased dosages meant decreased body gains as compared to controls. V. Hurst

## SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

**394. Equipment cleaning programs.** K. R. FOWLER, National Dairy Products Co., Inc., New York, N. Y. Ice Cream Trade J., **46,** 2: 60, 114. Feb., 1950.

Cleaning compounds should be weighed or

measured into bags and dispensed to the people doing the cleaning. One of the most economical ways of making up a cleaning solution is to use a centralized solution tank from which the heated solution may be piped to points of use and then dispensed through 0.25-in. hoses, with shut-off valves onto equipment to be cleaned. This method may save from $600–$4,000/yr., depending on the size of the plant.

A hot water generator or instantaneous system, in conjunction with temperature controls, water pressure regulators, 0.5-in. hose and shut-off valves will cut down fuel consumption as much as 50% over the common method of mixing steam and cold water. A water pressure of 25–40 lb. at the hose and a temperature of 115–120° F. will do an effective job of cleaning.

Phosphoric acid has been found more satisfactory for removal of milk stone than scouring powders. Sterilization is accomplished by using liquid chlorine, 10–15% available chlorine made up 220 ppm for spraying, 100 ppm for brushing and 50 ppm for flowing through pipes, pumps and vats. A spray gun with air pressure is the most effective means for sterilizing vats, tanks, coolers and other open surfaces.

Nylon-filled brushes have proved much more effective and less expensive in the long run to the conventional type of cleaning brushes.

W. H. Martin

**395. The cleaning and sterilization of milk plants.** A. Rowlands, Nat. Inst. for Research in Dairying, Univ. Reading, England. J. Soc. Dairy Technol., **2,** 3: 152–156. Apr., 1949.

The principles of cleaning and sterilizing dairy equipment are discussed along with the applications of various methods. The uses of steam, hot water, chlorine, alkalies and quarternary ammonium compounds are considered briefly.

E. M. Foster

**396. The importance of efficient washing of utensils for steam and hypochlorite sterilization.** A. H. Walters, C. M. Cousins and S. B. Edmunds. J. Soc. Dairy Technol., **2,** 3: 136–139. Apr., 1949.

A comparison of the bacteriological quality of milk produced on 2 farms during the summer of 1947 showed that high quality milk could be produced with either steam or hypochlorite sterilization of utensils provided the equipment was cleaned well before sterilization. One of the farms practiced machine milking, the other hand milking. Both used steam sterilization during the 1st half of the test period and hypochlorite during the latter half. Poor quality milk was produced only during the time when utensils were improperly cleaned before sterilization.

E. M. Foster

**397. Chemistry of cleaning farm milking equipment.** D. Levowitz, N. J. Dairy Laboratories, New Brunswick, N. J. Milk Dealer, **39,** 4: 104–107. Jan., 1950.

The chemistry of cleaning utensils used in handling milk is discussed to show that the practice of "rinsing milking utensils with cold water, directly after use" has needlessly complicated dairy farmers' lives and caused the production of oceans of poor milk; milk residues can be perfectly removed from utensil surfaces by dispersing them with appropriate wetting agent solutions, then flushing with plain water.

C. J. Babcock

**398. The sanitizing of milking machines.** A. C. Dahlberg, F. V. Kosikowsky, H. W. Seeley and A. A. Leuenthal. Cornell Univ., Ithaca, N. Y. J. Milk and Food Technol., **13,** 1: 5–18, 24. Jan.–Feb., 1950.

Good sanitizing results were obtained with neutral nonionic detergent and quaternary ammonium compounds when they were used with accepted sanitary procedures. When used together, they were not good solvents of dirt on milker rubber tubes. Quaternary ammonium compounds used alone or with nonionic detergents were not satisfactory in sterilizing unclean rubbers, but the combination gave good results when the rubbers were brush-washed in a hot solution. Cl solution was not as effective as lye when used as a solvent cleaner and sterilizer. The sterilization action of the alkaline and the quaternary compounds was increased by the alkaline solution. If a pH from 9.5–10.5 was not maintained in the washing solution, the quaternaries failed to destroy *Pseudomonas* organisms.

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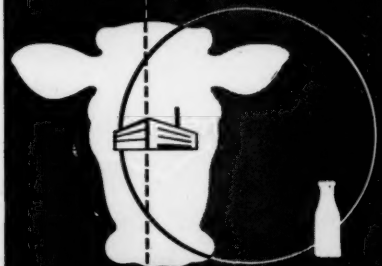

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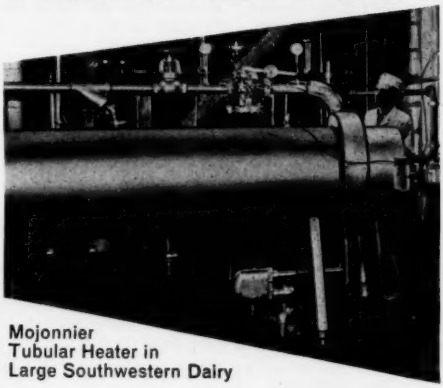

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